Microvascular Patterns in Experimental Spinal Cord Trauma: Effect of Mannitol

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1976

MICROVASCULAR PATTERNS IN EXPERIMENTAL SPINAL CORD TRAUMA: EFFECT OF MANNITOL

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B.A. Columbia University, 1971

Thesis submitted to the Faculty of Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

March, 1976

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April 12, 1976

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Acknowledgements

I am deeply indebted to Dr. William E. Allen, III of the Department of Neuroradiology and Dr. George J. Dohrmann of the Department of Neurosurgery for their warmth, guidance, and rapport in making this an informative and pleasurable endeavor.

The author wishes to thank Dolores G. Montoya for her surgical assistance, Barbara Hamill for her technical assistance, Gerald J. Conlogue for his assistance in obtaining the microangiograms, and Thomas S. McCarthy for his efforts in producing the photographs.

Dedication

To Susan for her love, patience, and support.

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cc.	cubic centimeters
CER	cortical evoked resonse; somatosensory evoked response
gm-cm	gram-centimeters
H-reflex	electrical correlate of the monosynaptic stretch reflex
kg	kilograms
KVP	kilovolt power
mA	milliamperes
mm	millimeters
NE	norepinephrine
pCO_2	partial pressure of carbon dioxide
pO_2	partial pressure of oxygen
T-5	fifth thoracic vertebrae
VPL	ventral posterior lateralis nuclei of the thalamus

Introduction

An injury to the spinal cord can be devastating in terms of both the physical and psychological difficulties experienced by the paraplegic or quadraplegic patient. Until recently, research in spinal cord trauma had experienced considerable neglect. This unfortunate lack of attention was due in part to a significant degree of pessimism and frustration on the part of both doctor and patient.

In 1965, the National Paraplegia Foundation estimated that there were about five adult paraplegics per 100,000 people living in the United States. The estimated incidence for new adult cases is 5,000 to 10,000 per year.

The average age of onset for traumatic paraplegia is 37 years and the average length of survival after such an injury is 12 years.

The economic losses from spinal cord injury to the nation as well as the individual are staggering. The average cost in 1967 for initial hospitalization per patient was reported to be $15,000. The cost of continued medical care was $2,000 per year. Therefore, the lifetime cost was estimated at between $40,000 and $50,000 per individual. The wage and tax loss was estimated at $240,000 for one patient or approximately 24 billion dollars for the spinal cord injured population.[36]

With respect to the incidence of automobile accidents,

the increased enthusiasm for potentially dangerous sports, the easy availability of hand guns, the involvement in wars, and the rising inflation rate, the magnitude of this problem is ever increasing. It is imperative for basic scientific research to define the pathophysiology of this injury and thereby, direct the way to more effective methods of treatment of the spinal cord-injured patient.

Since the mechanical damage incurred at the time of impact cannot be altered, attention has been directed toward halting the degree and progression of the secondary pathophysiological processes. Therapeutic modalities have usually been surgical, metabolic or a combination of the two. The resulting data has been inconsistent and disappointing concerning the response to these different forms of therapy. A better basis of understanding of the events occurring within the contused spinal cord along with more controlled studies that assess the efficacy of present forms of therapy is needed before meaningful methods of treatment can be discovered.

Purpose

Spinal cord swelling is due to both the central hematoma and vasogenic edema formation following trauma. The secondary effects of edema probably accentuate the damage following the initial destruction. Mannitol is an effective dehydrating agent. On a theoretical basis, it appeared ideal for interrupting the potentially devastating sequelae of edema and ischemia known to occur post-traumatically. If it was possible for mannitol to do this, the relationship of spinal cord microvascular changes and edema to subsequent functional recovery might be clarified; therefore this study was undertaken.

Hypertonic mannitol when injected intravenously is known to have a beneficial effect in reducing cerebrospinal fluid pressure and brain bulk; it has become an important aid in the treatment of cerebral edema. It has been observed by previous investigators that over the first hours following contusion of the spinal cord, the white matter becomes edematous. Intramedullary microvascular alterations and edema probably play important roles in the degree of functional deficit seen following contusion of the spinal cord. In a prior search of the literature an evaluation of the effects of mannitol on the microvasculature of the spinal cord following graded trauma could not be found.

The leptomeninges and dura mater encase the spinal cord such that swelling of the spinal cord causes an increased pressure. This increased tissue pressure probably contributes to the decreased perfusion states and ischemic sequelae seen post-traumatically. The need to reduce the spinal cord edema in order to break this theoretical cycle and regain or preserve neurological functions would therefore be obvious.

Utilizing a known experimental model for producing a standardized, quantifiable contusion, feline spinal cords, exposed by laminectomy were traumatized with a 500 gm-cm contusion, a force sufficient to render the animal permanently paraplegic. The relationship of vascular alterations and edema formation were investigated using high resolution microangiography and fluorescent blood flow studies through a comparison of untreated and mannitol-treated spinal cords. Certain parameters of spinal cord function were incorporated into the model such as the somatosensory cortical evoked responses which is a measure of conductivity in the ascending columns of the white matter.

Though the laminectomy required to produce this graded trauma violates the closed-space concept of the spinal canal by partially decompressing the spinal cord, this experimental trauma model is presently the only one in design that affords the benefit of reproducible quantifiable trauma.

Historical Review

> "One having a crushed vertebrae in his neck; he is unconscious of his two arms (and) his two legs and he is speechless. An ailment not to be treated."
>
> ...Edwin Smith Surgical Papyrus[10]

Experimental Model:

Until the development of an adequate experimental model, little progress had been made in elucidating the pathological processes occurring within the traumatized spinal cord. Early attempts at studying the traumatized spinal cord were very crude, using experimental models which were non-standardized and difficult to quantitate. Schmaus (1890) attached boards to the backs of vertically suspended rabbits and struck them.[61] Watson (1891) dropped dogs on their spines and examined the spinal cords following injury.[67] In 1899 Spiller studied the kitten spinal cord after the animal had been squeezed in a door.[62] In the early 1920's, some investigators produced an injury in dogs by exposing the spinal cord and squeezing it with their fingers[49,63] or scalpel handle.[49] After administering blows to the backs of rabbits with an iron rod, Ferraro (1927) examined the spinal cords using light microscopy.[35]

In 1911 Alfred Allen, in an attempt to determine the maximum amount of impact a spinal cord could receive and

still recover its function, designed an instrument whereby a given weight could be dropped through a vented guide frame from a known height on to the exposed spinal cord, thereby producing a given impact.[5] It was the first technique developed for delivering a reproducible, quantifiable contusion force to the spinal cord. The magnitude of each injury could be expressed in gram-centimeters, representing the product of the weight and the distance of the fall. Since the development of Allen's contusion device, little improvement has been made in the injury model over the ensuing years. Contemporary investigators in spinal cord trauma continue to use the same system with only minor modifications. Allen noted that an impact of 340 gm-cm produced a complete paraplegia of the spastic and transient type, and impacts of greater magnitude produce more severe and lasting symptoms.[4,5]

History of Research:

In addition to being pivotal in acute spinal cord injury research, Allen reported in 1908 the first comprehensive clinicopathological study of human spinal cord injuries, and treatment actually began under his influence. He emphasized the nature of central traumatic hemorrhagic necrosis and suggested the use of midline longitudinal myelotomy in order to drain the injured tissue of the products of edema and

hemorrhage. Nevertheless it was not until the work of Albin et al. in 1968 demonstrating the beneficial effect of hypothermia on the injured spinal cord that serious interest in spinal cord trauma research was initiated.[1] Since that time studies have focused upon alterations in morphology, metabolism, blood flow and neurophysiological responses as parameters of injury and recovery. New treatment modalities have been investigated in the wake of increased knowledge of the pathophysiology of this injury.

Pathology:

The traumatized cord is affected not only by direct mechanical injury at the time of impact, but after trauma certain pathophysiological changes occur which are believed to cause further damage. Some investigators have stressed the role of the initial mechanical factors, causing disruption of neuronal as well as vascular structures, as the critical parameters of injury.[1,40,53]

Others emphasize the progressive ischemia of the spinal cord post-traumatically as the principal secondary injury. Abnormal concentrations of neural transmitters have been postulated as primarily responsible for these vascular alterations.[6,29,32,39]

Certain authors have reported studies of the evolution and migration of edema.[59,66] Most investigators agree that

more than one of the above-mentioned post-traumatic changes in the spinal cord may be responsible for the resultant functional deficit; however, immediate as well as delayed alterations in the vasculature of the spinal cord appear to be major factors.

In order to clarify and define the pathophysiological processes that are occurring in the traumatized spinal cord, investigators have used a variety of techniques. Gross examination of the spinal cord following contusion showed subarachnoid hemorrhage. The spinal cord was swollen at the injury site and this swelling extended a small distance up and down the spinal cord. Softening of the spinal cord at the injury site was detectable after one hour. Transverse sections showed hemorrhage in the region of the gray matter.

Light microscopy studies of the spinal cord at various periods of time following spinal cord contusion have documented that the spinal cord lesion progresses with time.[28,37,65] After trauma there are usually multiple small hemorrhages seen in the central gray matter. The hemorrhages result from the rupture of thin-walled muscular venules with leakage of erythrocytes into the perivascular space. Within two hours the central hemorrhages have enlarged and by four hours after trauma much of the gray matter is replaced by hemorrhage.

From detailed ultrastructural studies, Dohrmann et al. noted that tears were seen in the walls of the muscular venules of the central gray matter.[18] Erythrocytes were seen in the perivascular spaces of the post-capillary venules and muscular venules of the gray matter and by one hour hemorrhage was noted within the neural tissue.

Vascular Morphology:

Microangiography was later used in studying experimental spinal cord injuries.[33,64] Fairholm and Turnbull, studying the microvasculature of the spinal cord following trauma, correlated the microangiographic and histological findings in corresponding areas of the original cord in lesions causing both complete and incomplete paraplegia.[34] At specified intervals following trauma, an arterial perfusion of an aqueous suspension of colloidal barium sulphate (micropaque) was begun before the animal was killed and continued post mortem. On the basis of their microangiographic findings, they divided the injured cord into two zones. Zone One corresponded to that part of the cord where there was no vascular filling with perfusate; it was characterized by complete necrosis of all elements located in the posterocentral part of the cord beneath and extending beyond the site of impact. Its size was dependent upon the degree

of injury. At four hours there was hemorrhagic necrosis of all structures in Zone One. Capillaries in Zone One appeared to progressively lose the ability to conduct perfusate during the first four hours.

Zone Two is that region surrounding Zone One. Microvascular patterns were normal in Zone Two, although neuronal and axonal degeneration was severe. The presence of patent blood vessels in Zone Two was thought to provide the potential for recovery of those neural structures in the spinal cord that were not irreversibly injured by the mechanical impact.

Allen et al.[6] using microangiographic techniques demonstrated the presence of focal areas of extravasation of contrast material in the gray matter immediately following spinal cord contusion, supporting the concept of immediate mechanical disruption of blood vessels. At 15 minutes following an injury sufficient to cause a transitory paraplegia there was a reduction in perfusion of peripheral arteries to the white matter, especially in the region of the dorsal columns. The blood vessels that were opacified appeared to be reduced in caliber. The central arteries appeared morphologically normal but there was less perfusion of the arterial network of the central gray matter. Thirty minutes to two hours following the injury the perfusion of both the white and gray matter improved and the caliber of the blood vessels approached normal.

Electrophysiology:

Donaghy and Numoto found that they could predict the reversibility or irreversibility of spinal cord contusions in experimental animals by measurements of sensory evoked potentials.[23] D'Angelo, Van Gilder and Taub using graded trauma found that the return of the evoked response could be correlated with the severity of the pathological damage to the spinal cord.[14]

In transitory traumatic paraplegia (a 300 gm-cm injury) there is an immediate loss of somatosensory cortical evoked response, a measure of white matter function, which gradually returns within one to two hours after injury.[14] The H-reflex, the electrical correlate of the monosynaptic stretch reflex, is a measure of the integrity of the gray matter.[13] The absence of this segmental reflex after trauma indicates considerable disruption of the central internuncial pool by traumatic hemorrhage. The H-reflex disappeared within two hours following a transitory injury. In permanent traumatic paraplegia (a 500 gm-cm injury) the somatosensory cortical evoked response and H-reflex immediately disappeared and did not return during the first four hours.

Allen et al.[6] showed that the immediate loss of spinal cord conduction in the white and gray matter occurred in the presence of microvasculature that was morphologically normal; however, the ensuing functional alterations did

correlate with the integrity of the intramedullary vasculature.

Fairholm and Turnbull concluded that degeneration of neural structures occurred in the absence of microvasculature disruption (Zone Two).[34] Allen noted vasoconstriction and stasis within the first 30 minutes following trauma with gradual improvement of vascular filling over the ensuing two hours of observation. Although transient, the diminished flow was thought to produce sufficient ischemia to cause permanent neuronal damage.

Blood Flow Patterns:

Dohrmann et al.[17,20] reported that the intravenous injections of a fluorescent dye, Thioflavine S, was an effective way of demonstrating the blood flow patterns in the intrinsic vessels of the spinal cord. The substance stains the walls of blood vessels through which it passes; thus when studied under ultraviolet light, blood vessels in which flow was occurring will fluoresce. If Thioflavine S is administered by rapid intravenous injection and the spinal cords are excised within one circulation time and examined under ultraviolet light, it is possible to determine in which vessels blood was flowing at the time of injection. In this manner, alterations in spinal cord blood flow patterns at various intervals following injury were determined.

Dohrmann et al. showed that the entire gray matter became hemorrhagic with no evidence of perfusion within the first four hours following contusions of varying severity.[21,22] In transitory traumatic paraplegia, a complete paraplegia which resolves without any therapeutic intervention other than good nursing care, white matter blood flow studies demonstrated a marked decrease in the number of vessels perfused at 15 minutes following impact. At 30 minutes many of the vessels had evidence of renewed blood flow. There was a secondary decrease in the fluorescent vascular pattern at one hour followed by progressive improvement. The transitory paraplegia which was noted clinically was postulated to have been caused by direct neuronal injury from the constusion force and/or probable ischemic injury to the white matter secondary to transient alterations in blood flow patterns.[30] In studies of more severe spinal cord trauma(permanent traumatic paraplegia), the early transient reduction in perfusion of peripheral white matter also occurred; however, in contrast to the transitory lesion, the decrease in blood flow patterns continued after one hour. At 24 hours, although the gray matter remained in a state of hemorrhagic non-perfusion, the blood flow patterns in the white matter resembled those of the control group. A correlative study of spinal cord blood flow patterns and microangiography by Dohrmann and Allen suggested that vaso-

spasm occurs in the white matter following trauma, and that this was largely responsible for the decreased vascular perfusion of the white matter. Their studies seem to indicate that the turning point in the progression of impaired vascular perfusion in the white matter occurs by one hour post contusion.[16]

In experiments on the contusion of canine spinal cords, Kelly et al. found a dramatic fall in tissue pO_2 of the injured segment.[43,44] Ducker and Perot measured local spinal cord blood flow directly by Xenon 133 desaturation curves after microintramedullary isotope injections and found a progressive decrease in blood flow within the spinal cord at one and two hours after trauma.[30] Locke et al. noted a rise in the lactate content of the spinal cord after injury; this was present in supra-normal concentrations for up to 40 hours. The authors noted that tissue lactate rose as blood flow in the spinal cord decreased. They postulated that local spinal cord ischemia/hypoxia play a role in the genesis of the post-traumatic spinal cord lesion.[48]

Catecholamines:

An increase in the norepinephrine (NE) content of the spinal cord following contusion was reported by Osterholm and Mathews. By blocking NE synthesis within 15 minutes after contusion, the increase in intraspinal NE and the devel-

opment of hemorrhage was reported to be inhibited. Excessive amounts of NE were postulated to cause vasospasm and ensuing hemorrhage.[54] Osterholm, et al. noted increased levels of serotonin in the CSF as well as within the injured tissues and postulated that following cerebral trauma, serotonin is displaced from an intracellular site and in its free state acts on other neurons causing neurological deficits, as well as acting upon capillaries and venules to produce cerebral edema.[54] Other investigators, however, have not been able to confirm these findings.[11,15,40,50]

In summary, the susceptibility of the central gray matter to trauma was first emphasized by Allen in 1914. It is now well known that small gray matter hemorrhages progress to a central hemorrhagic necrosis. It has been demonstrated that trauma leads to vascular spasm in the white matter, possibly as a result of mechanical forces and secondarily through the action of spasmogens which may be released from the blood in the subarachnoid space.[42] Spasm can lead to ischemia and anoxia and subsequent permanent neural damage could ensue.

Trauma to the spinal cord may result in paraplegia because of anatomical disruption and/or deformation of the neural elements or because of a compromise of the blood supply to the spinal cord. Disruption of the neural elements is irreversible because of the inadequate potential for

regeneration in the central nervous system.

Less severe injuries that cause either paraparesis or a transitory paraplegia may well be secondary to vascular factors.[8,25,26] Recovery that is seen in transitory paraplegia implies that the integrity of the traumatized spinal cord has been maintained and reestablishment of normal neural metabolism probably has occurred.[8] Assenmacher and Ducker found that with irreversible injury to the spinal cord neural recovery was precluded primarily because of the vascular changes which cause progressive hypoxia. The circulatory disturbances become worse with time; these alterations included intravascular stasis, venous dilation, marked intramedullary hematoma formation, and edema of the cord. In five days the necrosis of the injured segment of the spinal cord was noted. Maintenance of the spinal cord circulation by either pharmacological, operative or other therapy is therefore essential to the functional recovery of the contused spinal cord.

History of Treatment:

Since the mechanical damage incurred at the time of impact cannot be altered, attention has been directed toward halting the degree and progression of the secondary pathophysiological processes. Experimental treatments have usually been surgical, metabolic or a combination of the two.

Therapeutic modalities have included midline posterior myelotomy, rhizotomy, hyperosmolar solutions, hypothermia, steroids, antifibrinolytic agents, hyperbaric oxygen, methylsergide, alpha-methyl tyrosine, reserpine, alpha-methyl dopa, phenoxybenzamine, disulfran, and 6-hydroxydopamine. The rationale for certain of the therapeutic approaches follow.

Allen proposed the use of midline myelotomy for early treatment of severe cord injury as a means of mechanical decompression and removal of noxious blood elements.[4,5] Osterholm has suggested the use of rhizotomy as a means of interrupting what he believes to be the afferent loop for traumatic hemorrhagic necrosis.[51,55] Since edema results following spinal cord injury, dehydration was thought to lessen neuronal destruction. Joyner and Freeman following encouraging results suggested the use of urea.[51] Deep hypothermia (7°- 20°C) was tried because it slows neural enzymatic processes and reduces cellular metabolic rates and oxygen requirements.[1,2,68] Steroids were used to maintain vascular integrity after injury, to protect cellular membrances during hypofusion states, and to support lyzosomes.[7,27] Previous clinical trials have shown its efficacy in intracranial edema, reducing intracranial hypertension and neurological deficits. Campbell et al. suggested the use of epsilon-aminocaproic acid, an antifibrinolysin, to arrest

the hemorrhage within the traumatized spinal cord.[12]

Since spinal cord pO_2 rapidly declined within the injured area of trauma, Kelly et al. found local atmospheric hyperbaric treatments lead to a significant functional recovery.[44]

The resulting data has been inconsistent concerning the response to these different forms of therapy. Some of the data indicates that the extent of reversibility of spinal cord injury under these experimental conditions are determined at the time of impact and demonstrate no evidence of significant secondary injury amenable to post-traumatic manipulation by the treatment regimens studied. Others have found improvements in vascular perfusion and/or neurological testing following the administration of one therapeutic regimen alone or in combination. The number of inconsistent findings from various laboratories may well be related to a lack of standardization of the trauma model between different laboratories and the difficulty in evaluating the neurological status of the experimental animals. A better basis of understanding of the events occurring within the contused spinal cord is needed before meaningful methods of treatment can be discovered.

Experimental Data

A Correlation of Intramedullary Blood Flow Patterns and Microangiography in Experimental Spinal Cord Trauma: Effect of Mannitol Therapy

Feline spinal cords, exposed by laminectomy at the T-5 level, were traumatized with a 500 gm-cm contusion, a force sufficient to render the animal permanently paraplegic. A fluorescent staining technique that depicts spinal cord perfusion patterns and high resolution microangiography which depicts vascular morphology were utilized to study the relationship of microvascular alterations and edema following a standard contusion. Mannitol was given at one hour following impact and at specified intervals of one and one-half hours, two hours, three hours, and four hours following contusion; the mannitol-treated spinal cords were compared to the untreated spinal cords. Blood pressure and cortical evoked responses were followed as parameters of injury and/or recovery. Temperature and end-expiratory pCO_2 were monitored and maintained within normal range and reflected the stability of the experimental model.

Previous investigators had observed that over the first hours following contusion of the spinal cord, the white matter becomes edematous. Green and Wagner using Evans blue bound to albumin, a fluorescent indicator, studied the development of the post-traumatic edema in the spinal cord and described it as evolving in the gray matter at one hour

post-contusion and migrating to involve all the white matter in the region of injury by eight hours.[38] Yashon et al.[70] reported an increase in the water content within five minutes in the traumatized segment, which persisted for 15 days. Richardson and Nakamaura in their electron microscopic study of spinal cord edema noted thickening of the capillary wall, swelling of the astrocytic processes and enlargement of the extracellular space of the white matter.[59] They noted that mannitol had its primary effect on the endothelium and astrocytic processes with an increase in the electron density of both and a significant decrease in the volume of the latter. Parker et al.[56] had studied the effects of mannitol on spinal cord trauma after giving a blow with an iron rod to the lumbar vertebral column. By using myelography, he found a 15% to 25% reduction in the diameter of the spinal cords of mannitol-treated animals relative to the untreated animals.

Spinal cord swelling is due to both the central hematoma and edema formation. The leptomeninges and dura mater encase the spinal cord such that swelling of the spinal cord causes an increased tissue pressure. The increased tissue pressure probably contributes to the sludging, stasis, decreased perfusion states, and ischemic sequelae seen post-traumatically. The need to reduce the spinal cord edema in order to break this theoretical cycle and regain or preserve neurological

function would therefore be obvious. Can mannitol serve this purpose? The purpose of this study is to investigate and correlate the possible changes in microangiographic and fluorescent vascular pattern after the administration of mannitol in experimental spinal cord trauma.

Materials and Methods

Adult cats weighing three to four kg. were anesthetized with intraperitoneal sodium pentobarbitol (35 mg/kg). A no. 190 polyethylene catheter was introduced through the right femoral vein for intravenous administration of medications and a similar catheter was introduced through the right femoral artery into the abdominal aorta for monitoring the arterial blood pressure via a Statham strain gauge and a Grass Model 6 polygraph. Animals which were to undergo microangiographic studies had their right common carotid exposed and cannulated with a no. 205 polyethylene catheter which was advanced into the proximal aortic arch for later use in the perfusion of contrast material. The animal was then intubated and respirations were controlled with a Harvard small animal respirator after muscle paralysis was achieved by a 20 mg intravenous injection of gallamine triethiodide (flaxedil). Supplemental doses of flaxedil were given occassionally to maintain adequate relaxation.

Temperature was monitored by a rectal probe attached to a Yellow Springs Instrument Company Model 73 Telethermometer and maintained between 37° and 39° C using a heating pad under the animal's abdomen. Through a small opening in the anesthesia tubing, a catheter was advanced into the endotracheal tube and connected to a Beckman Gas Analyzer Model LB1 and a microcatheter sample pump and pick up to constantly

monitor the end-expiratory pCO_2. By changing the respiratory rate and/or tidal volume, the end-expiratory pCO_2 could be maintained at 2 to 4 volumes per cent.

After placing the animal in a sterotaxic frame a midline scalp incision was made; the temporalis muscle was freed from its origins and the overlying periosteum was removed from the right side of the skull. The coronal sutures were identified and a screw electrode was placed in the right frontal skull over the primary somatosensory area of the brain. A reference electrode was placed in the frontal sinus(Fig. 1.). Stimulating electrodes were placed on the contralateral posterior tibial nerves and a 0.1 m sec. pulse of lv/sec. was used. 64 successive cortical transients were averaged on a Nicolet Instrument Corporation Model 1074 computer for analysis. The analysis time was 0.16 seconds. Photographs of the computer-averaged cortical evoked response (CER) were taken with a Tektronix C-5 oscilloscope camera. Cortical evoked responses averaged before and after surgical exposure of the cord served as controls. CER's were recorded before trauma, immediately after trauma, and at hour intervals up to four hours after injury.

A supraspinal midthoracic incision was made exposing the erector spinal muscles. The paraspinal musculature was freed from its insertion on the spine with the aid of periosteal elevators and later retracted exposing the

spinal column. A laminectomy was performed exposing the 5th thoracic segment of the spinal cord and leaving the dura mater intact. The spinal cord was traumatized using a modification of the apparatus described by Allen[9] and Albin[11] et al.: a 20 gm tungsten weight was dropped a distance of 25 cm through a perpendicularly oriented plastic tube directly onto an impounder resting on the posterior spinal cord (Fig. 2.). Vertical orientation of the tube was checked by a small bubble balance mounted on the top of the tube. The weight (gm) multiplied by the height (cm) determined the contusion (500 gm-cm).

Blood Flow Pattern Study:

A total of eighteen cats were used. Eight cats were traumatized and injected with Thioflavine S (as below) at one and one-half hours, two hours, three hours and four hours after impact. A non-traumatized cat served as a control animal. Eight additional cats were given a 15 cc/kg dose of 20% solution of mannitol (3 gm/kg) at the one hour interval following trauma, and were injected with Thioflavine S at one and one-half hours, two hours, three hours and four hours. One cat which had undergone a laminectomy only also received mannitol and served as a control. At one of the specified time intervals following contusion, the animals were given rapid (two - four sec.) injection of 4% Thioflavine S

(1 cc.kg, 37°) into the femoral vein. Thioflavine S is a fluorescent dye which stains the walls of blood vessels without damaging them. When injected intravenously it will selectively stain vessels through which it has passed. The exposed spinal cord was then excised within one circulation time (10 sec.), flash frozen and cut into sections 150 microns thick on a cryostat microtome. Photographs of cut sections were taken under ultraviolet light using a BG3 filter and a Zeiss Epi-technoscope equipped with a 35 mm camera. Kodak Ektachrome film was used. Exposure time was 60 seconds.

Microangiographic Study:

A total of eighteen cats were used. Eight cats were traumatized and infused with a micropaque-gel suspension at one and one-half hours, two hours, three hours and four hours after impact. A non-traumatized cat served as a control animal. Eight additional cats were given a 15 cc/kg dose of a 20% solution of mannitol (3 gm/kg) at the one hour interval following trauma, and were injected with the micropaque-gel suspension at one and one-half hours, two hours, three hours and four hours. One cat which had undergone a laminectomy only also received mannitol and served as a control. At one of the specified time intervals following contusion, the animals were infused through the carotid catheter with 50 cc of 75% micropaque-gel suspension pre-

heated to 37°C. The infusion was controlled to maintain but not to exceed the pre-trauma systolic blood pressure. As the infusion was begun cardiac function was stopped with an intravenous injection of concentrated KCl solution. At the completion of the infusion, the spinal cord was frozen *in situ* with liquid nitrogen, removed with the spinal column using a Stryker saw and rib cutters, and placed into a -20°C freezer for one hour to ensure solidification of the Barium-gel suspension, and then fixed in 10% formalin. After a ten day period of fixation, the spinal cord was dissected from the vertebral column. The dura was removed and the specimen was radiographed in the antero-posterior and lateral projections. Three millimeter thick sections were then cut, beginning with the 3 mm of the traumatized portion and then radiographed.

The radiographic unit consisted of a water-cooled type 0-2 L diffraction tube with a thin beryllium window and a chromium copper target with a 0.2 x 1.0 mm focal spot. Exposures in the range of 20 KVP and 20mA were used varying the time depending upon the thickness of the specimen. The intact spinal cord, approximately 7 mm in thickness, required between five to six minutes of exposure time, and the cross-sections three minutes for optimum exposure. Kodak type R film was used. The specimen, sealed in plastic(Fig. 3.), was placed into a receiver which was fitted to the bottom

of the extension cone with a focus-to-field distance of 70 cm(Fig. 4.). The radiographs were processed by hand in D19 developer at 68° for eight minutes; fixing time was double developing time and washing time was double the fixing time.

Under light microscopy, the microangiographic pattern of the spinal cord was evaluated in the antero-posterior and lateral projections, as well as in cross-section. Areas proximal and distal to the site of trauma were assessed in order to document the adequacy of the infusion in each animal and to serve as a standard of comparison.

Results

Physiological Monitoring

Following laminectomy, feline spinal cords were traumatized with a 500 gm-cm contusion at the T-5 level. Blood pressure and cortical evoked responses were followed as parameters of injury and/or recovery. A catheter was introduced through the right femoral artery into the abdominal aorta for constant monitoring of the arterial blood pressure via a Statham strain gauge and a Grass Model 6 polygraph. A screw electrode was placed in the right frontal skull over the primary somatosensory area for recording the CER following stimulation of the contralateral posterior tibial nerves. A reference electrode was placed in the frontal sinus. These responses were averaged on a computer and photographs of the computer-averaged cortical evoked responses (CER) were taken with an oscilloscope camera. CER's were recorded before trauma, immediately after trauma and at hour intervals up to four hours after injury. Temperature and end-expiratory pCO_2 were under constant monitoring and maintained within normal range and reflected the stability of the model.

Blood Pressure:

Following trauma to the spinal cord the characteristic pressor response occurred as systolic levels above 300 mm Hg

were often noted (Fig. 5A.). Generally it reflected an increase of about 90 to 130 mm Hg above pretrauma systolic levels. This pressor response is preceded by a latent period of two to six seconds following trauma.

A period of hypotension of approximately 60 to 70% of pretrauma levels immediately followed the hypertensive phase and persisted for 15 to 30 minutes and then began a gradual climb toward pretrauma levels over the following hour (Fig. 5A.).

During the infusion of mannitol an increased pulse pressure was noted as the circulating plasma volume expanded (Fig. 5B.). During this interval an increase in systolic blood pressure amounting to as much as 20 to 4o mm Hg and an increase in diastolic blood pressure between 10 to 20 mm Hg over pre-mannitol blood pressure levels developed. Within 30 to 60 minutes following infusion diuresis occurred and a gradual decline toward pre-mannitol blood pressure levels was noted.

Cortical Evoked Responses:

In permanent traumatic paraplegia following the 500 gm-cm contusion, the somatosensory cortical evoked response (CER) immediately disappeared and remained absent for the remaining four hours of observation in both the untreated and the mannitol-treated groups (Fig. 6.).

Microangiography and Blood Flow Patterns

Animals which were to undergo microangiographic examination were injected with 50 cc of a 75% micropaque-gel suspension through a carotid catheter at an infusion pressure which did not exceed pretrauma systolic blood pressure. The spinal cords were frozen *in situ* with liquid nitrogen to ensure solidification of the Barium-gel suspension before fixation in 10% formalin and radiographing. Animals which were to undergo fluorescent staining studies were injected through the femoral vein with one cc/kg of a 4% solution of Thioflavine S and had their cords excised within one circulatory time (ten seconds), frozen on a cryostat and later examined under fluorescent microscopy. The spinal cords of these two groups were compared at one and one-half hours, two hours, three hours and four hours following trauma.

Observations of the normal non-traumatized spinal cords:

Gray Matter - At two hours following laminectomy the untreated and mannitol-treated non-traumatized spinal cords displayed a normal vascular pattern. The gray matter was supplied predominantly by a rich arterial network arising from the central arteries as well as the penetrating branches of the posterior spinal arteries supplying the posterior horns (Fig. 7.).

Under ultraviolet light the spinal cord exhibited a pale blue-green autofluorescence. Thioflavine S

fluoresced yellow. The sulcal arteries fluoresced deeply and the gray matter showed a more concentrated array of vessels. There was essentially no detectable difference between the fluorescent vascular pattern of the spinal cord of the mannitol-treated and untreated animal (Fig. 16.).

White Matter - The surface vasculature consisting of the anterior spinal and paired posterior spinal arteries and their interconnecting pial anastomosis were intact and of normal caliber(Fig. 7A. & 7B.) There was a rich anastomic network of peripheral arteries arising from the surface vessels supplying the underlying white matter (Fig. 7C.) A border zone was noted where there was an overlap of supply to the white matter circumjacent to the gray matter by the centrifugal (central) and centripedal (peripheral) system.

Many vessels of the white matter stained with Thioflavine S and radiated from the periphery toward the center of the cord(Fig. 16.).

Experimental Group:

#1 Laminectomy-Trauma

A laminectomy was performed at the T-5 level. A 20 gm weight was dropped a distance of 25 cm through a perpendicularly oriented vented plastic tube onto an impounder resting on the exposed spinal cord. The

animals therefore received a 500 gm-cm contusion, sufficient to render them permanently paraplegic. Following injection of the micropaque-gel suspension or Thioflavine S, the spinal cords were studied as previously described.

Observations of the spinal cord one and one-half hours post-trauma:

Gray Matter - Severe narrowing was noted in the posterior spinal arteries and to a much lesser extent in the anterior spinal artery. The perforating branches of the posterior spinal arteries were noted to be moderately decreased in number and there was severe narrowing of the vessels throughout the gray matter. Moderate focal extravasation throughout the dorsal columns and their immediate base was manifested by large areas beginning to coalesce (Fig. 8.). This was also shown in the Thioflavine S specimens as large non-fluorescent hemorrhagic foci appearing dark red or black in the central gray matter (hemorrhagic non-perfusion).

White Matter - Some focal extravasation of contrast in the circumjacent white matter was noted. There was diffuse severe narrowing of the peripheral vessels of the white matter. A moderate decrease in the number of peripheral vessels was noted (Fig. 8.).

Thioflavine S studies showed multiple hemorrhagic foci in the periphery. There was a marked decrease

in fluorescence in the periphery, most notable in the posterior columns compared to controls. Subdural hemmorhage was noted.

Observations of the spinal cord two hours post-trauma:

Gray Matter - The caliber of the surface vessels was much improved over the 1½ hour group, showing only a mild degree of narrowing(Fig. 9A. & 9B.). The perforating branches of the posterior spinal vessels were decreased in number and displayed a mild degree of narrowing. The central gray matter was practically totally replaced by contrast (Fig. 9C.).

Thioflavine S revealed that the central gray matter had a larger, more extensive region of hemorrhagic non-perfusion as compared to that at one and one-half hours following contusion (Fig. 16.).

White Matter - There was essentially no filling of the peripheral vessels supplying the white matter. There were multiple small foci of hemorrhagic non-perfusion in the posterior columns and the remainder of the peripheral white matter. A slight improvement in the number of fluorescent vessels in the periphery was noted as compared to the one and one-half hour group; however, the perfusion of the posterior columns was markedly decreased(Fig. 16.).

Observations of the spinal cord three hours post-trauma:

Gray Matter - Narrowing of the penetrating branches of the posterior spinal arteries was noted. Focal areas of extravasated contrast were noted throughout the gray matter (Fig. 10.). The entire gray matter was hemorrhagic and had no evidence of perfusion with Thioflavine S (Fig. 16.).

White Matter - Swelling at the injury site was noted. Small focal areas of extravasated contrast were noted within the immediate white matter adjacent to the central matter. The peripheral vasculature approached a normal distribution, though reduced in caliber.

There was a total lack of fluorescing vessels in the posterior columns, in addition to multiple foci of hemorrhages. Circumferentially, there was a marked increase in the non-fluorescing area of the peripheral white matter.

Observations of the spinal cord four hours post-trauma:

Gray Matter - Extravasation of contrast was not noted centrally. Only the major branches of the central and posterior arteries were filled; the smaller branches to the gray matter and dorsal columns did not fill (Fig. 11.). Virtually no fluorescing vessels were noted (Fig. 16.).

White Matter - The spinal cord was swollen at the trauma site. There was sparse filling of the white

matter, particularly within the border zone between the white and gray matter (Fig. 11C.).

Multiple small foci of hemorrhagic non-perfusion were seen in the posterior columns. Virtually no fluorescence was present in the white matter (Fig. 16.).

Experimental Group:

#2 Laminectomy - Trauma - Mannitol

The animals in this experimental group were similarly traumatized, receiving a 500 gm-cm contusion at the T-5 level; however, these animals received a 15 cc/kg dose of a 20% solution of mannitol (3gm/kg) at the one hour interval following trauma. They were subsequently studied for microangiographic and blood flow pattern changes at one and one-half hours, two hours, three hours and four hours following trauma.

Observations of the spinal cords one and one-half hours post-trauma(30 minutes post-mannitol):

Gray Matter - A paucity of vessels was noted. There was filling of only the larger branches of the posterior and anterior spinal arteries. There was practically no filling of the central gray arterial network. Very minor narrowing of the surface vessels was noted. The extravasation of contrast material was absent.

Thioflavine S demonstrated that the area of central hemorrhagic non-perfusion had become larger than that

of the untreated animals at this time.

White Matter - Very few peripheral vessels filled with contrast; however, these opacified vessels were not narrowed(Fig. 12.).

There were small foci of hemorrhagic non-perfusion mainly in the posterior columns. In general, there was no detectable improvement in the fluorescent vascular pattern as compared to the untreated animals at this time interval following trauma (Fig. 16.).

Observation of the spinal cord two hours post-trauma (one hour post-mannitol):

Gray Matter - The surface vessels were normal and unremarkable. There was filling of only the major branches of the anterior and posterior spinal arteries. Extravasation and narrowing were not noted (Fig. 13.).

The large area of hemorrhagic non-perfusion was seen; it was smaller than in the one and one-half hour treated group.

White Matter - Microangiograms showed an almost total absence of filling of peripheral vessels within the white matter (Fig. 13.). Multiple foci of hemorrhagic non-perfusion were seen in the posterior and lateral columns. An increased fluorescent vascular pattern of the spinal cord was present relative to the two hour untreated spinal cords. Fluorescent vessels could be

seen radiating from the periphery centrally, though to a much lesser extent in the posterior columns(Fig. 16.).

Observations of the spinal cord three hours post-trauma (two hours post-mannitol):

Gray Matter - Mild narrowing of the anterior and posterior spinal arteries was noted. There was no filling of the penetrating branches of the anterior and posterior spinal arteries. A few small focal areas of extravasation were noted (Fig. 14A,B,C.)

Hemorrhagic non-perfusion of the central matter remained the pattern.

White Matter - Very little peripheral white matter vasculature filled with contrast (fig. 14C.).

A decrease in the number of areas of hemorrhagic non-perfusion in the peripheral white matter was noted. A marked increase in the number of fluorescing vessels radiating from the periphery towards the center of the spinal cord was noted. The fluorescent vascular pattern of the lateral white matter approximated normal in both distribution and richness of array. The improved blood flow patterns were in striking contrast to the virtual lack of fluorescence seen in the untreated animals. The dorsal column fluorescent blood flow pattern was essentially unchanged as compared to the two hour time periods(Fig. 16.).

Observation of the spinal cord four hours post-trauma (three hours post-mannitol):

Gray Matter - The surface vessels were of normal caliber. There was filling of only the major branches of the posterior and anterior spinal arteries. A small area of extravasation of contrast was noted centrally (Fig. 15C.).

The central gray region remained in a state of hemorrhagic non-perfusion; no fluorescing vessels were seen(Fig. 16.).

White Matter - Vascular narrowing was absent and there was slightly more filling of the peripheral system. There was a decrease in the number of small focal regions of hemorrhagic non-perfusion in the white matter.

Fluorescence of many vessels of the anterior and lateral columns was noted. A slight increase in the number of fluorescent vessels in the white matter, as compared to the three hour group, could be seen radiating from the periphery to the central portion of the cord. There was no improvement in the fluorescent vascular pattern of the posterior columns relative to the previous time period. A striking improvement was apparent in the number of fluorescent vessels as compared to the untreated animals (Fig. 16.).

Discussion

Physiological Monitoring:

Blood Pressure:

The pressor response following spinal cord trauma has previously been described and is often referred to as the "Cushing Reflex."[3,31,58] The primary event in the hypertensive phase is thought to be the activation of sympathetic preganglionic cells in the cord by mechanical deformation, rather than anoxia, adrenal catecholamine release or activation of specific baroreceptors in the spinal canal. The hypotensive phase, which follows the initial hypertension was postulated to be related to a subsequent absence of sympathetic vascular tone.[58] The alteration in systemic blood pressure in both components of this response is thought to be of significance in the ensuing progressive post-traumatic spinal cord dysfunction, where changes in the autoregulation of the spinal cord blood flow is known to occur.[39]

It is generally accepted that most hypertonic solutions when given in large doses rapidly mobilize tissue fluids to such an extent that the circulating plasma volume becomes greatly increased in a matter of minutes. The elevated systolic and diastolic blood pressure levels as well as the wider pulse pressure noted after the infusion of mannitol

probably reflects this phenomenon.[69]

Cortical Evoked Response:

Donaghy and Numoto applied somatosensory cortical evoked response (CER) to experimental trauma as a parameter for predicting recovery. Since that time CERs have been measured in studies on experimental spinal cord trauma. The effects of graded trauma on the CER have been previously described by D'Angelo et al..[14]

The CER is a good measure of white matter function. Though the mechanism inhibiting conduction following spinal cord impact injury is essentially unknown, mechanical distortion or disruption of fibers, ischemia, hypoxia or neurochemical depression at the site of injury have been implicated as possible causes of these conduction changes. An absent CER immediately following impact has been shown to occur in the presence of a microvasculature that was essentially morphologically normal.[6] Dohrmann et al. demonstrated no change in the blood flow pattern of the white matter at five minutes following contusion.[22] Assenmacher and Ducker from direct observation of the pial microcirculation found no evidence of vasoconstriction in the immediate post-trauma period.[8] The return or disappearance of the evoked response following impact could be correlated with the severity of the spinal cord damage, specifically, posterior column damage.[26] The data suggests that the loss

of conduction in the white matter of the posterior columns in the immediate post-traumatic period does not appear to be related to a detectable ischemic process.

In both the untreated and mannitol-treated cords the CER did not return over the first four hours following trauma. Similar changes in the CER in permanent traumatic paraplegia (500 gm-cm contusion) have been noted by others.[6,14] This absence of the CER and its lack of return may well relate to the ensuing pathophysiological changes occurring within the first hour after trauma.

Microangiography and Blood Flow Patterns:

Contusion of the spinal cord results in morphological, physiological, biochemical, and vascular alterations. As the pathophysiological processes evolve following trauma, alterations in the microvasculature are occurring. The delayed changes in the microvasculature following trauma do, however, correlate with the progressive loss of conduction within the gray matter at the site of injury.[6]

Hemorrhage into the gray matter following spinal cord trauma has been demonstrated by histopathologic studies. These hemorrhages coalesce and eventually replace the gray matter at the site of injury. During the time of hemorrhage into the gray matter, spinal cord edema begins to develop. It appears to spread from the gray matter centripetally

into the white matter. The destruction secondary to the hemorrhages and contused nerve fibers become accentuated by the secondary edema compressing the nerve fibers, particularly when the swollen cord is compressed against the dura. If this process continues without interruption, degeneration of the myelinated fibers of the white matter can occur. Intramedullary microvascular alterations and edema probably play important roles in the degree of functional deficit seen following contusion of the spinal cord. Following trauma ultrastructural changes were noted in the microvasculature, perivascular spaces and myelinated nerve fibers of the spinal cord.[19,59]

The relationship of vascular alterations and edema formation were investigated in this study using microangiography and blood flow studies. The decrease in the edema of the spinal cord and a subsequent decrease in spinal cord compression had a beneficial effect on the perfusion of the intrinsic microvasculature. Microangiography provided information concerning the static state of the blood vessels of the spinal cord(Tables 1-3). This technique demonstrated the vascular integrity, shape, and/or patency with a given perfusate at a given perfusion pressure. Thioflavine S, on the other hand, afforded a dynamic study of the intramedullary perfusion pattern. Although microangiography and blood flow pattern studies do not necessarily depict

quantitative data concerning blood flow or perfusion, a correlation of the two techniques appears to give a more accurate assessment of the vascular response following trauma than either one independently. This was shown in a recent study by Dohrmann and Allen through a similar correlation of microangiographic and blood flow studies in the spinal cord in transitory and permanent paraplegia. They postulated that the turning point in the progression of impaired vascular perfusion in the white matter occurred by one hour post contusion.[16] Allen had previously noted significant vasospasm at the one hour interval which correlated well with the studies of Brodner, Dohrmann, and Rubin who noted peak CSF serotonin levels at the same time interval.[6,11] Kapp et al.[42] had noted that spasm secondary to the initial mechanical injury lasts only 15 minutes. Dohrmann and Allen noted that the decrease in perfusion of the white matter at the one hour interval appeared to either stabilize and return to normal as seen in transitory paraplegia or continue decreasing and return by 24 hours, by which time irreversible damage had occurred to the major sensory and motor tracts.

In this study cats were given mannitol one hour after sustaining a 500 gm-cm contusion to their thoracic spinal cords. At specified intervals thereafter, the spinal cords of the mannitol-treated animals were compared with the untreated spinal cords at similar intervals. Although

the Thioflavine S studies had shown that mannitol had a beneficial effect on intramedullary perfusion of the white matter in the contused spinal cord, the microangiography had shown similarly treated spinal cords to have a near total absence of extravasated contrast material centrally, filling of only the major vessels of the gray matter, and marked paucity of the peripheral white matter and border zone region (Tables 1 & 2). This pattern of distribution implied that only vessels of a certain caliber were being perfused or filled with contrast. It was apparent that this marked paucity in the vascular filling among the mannitol-treated spinal cords did not correlate with the striking improvement seen in the blood flow pattern studies, and was in some way related to the treatment.

The decreased vascular filling with contrast so ubiquitous among the mannitol-treated spinal cords was initally considered to be the result of a mechanical blockage to the passage of perfusate. Since this phenomenon was unique to the injury site and since cross-sections taken proximal and distal to the trauma site displayed a normal pattern of vascular filling of small vessels, microthrombi were thought to be contributory. The blood flow pattern studies, with marked improvement in the fluorescent vascular pattern at the later time intervals would not support this hypothesis.

Rosomoff had noted changes in the distribution of

intracranial water following the administration of hypertonic urea. He noted that the blood volume more than doubled, while the CSF fluid volume increased and CSF pressure decreased. This phenomenon is consistent with the findings of an apparent increase in the number of vessels perfused in the blood flow pattern studies and the decreased narrowing noted in the microangiographic studies among the mannitol-treated spinal cords.

Perhaps the apparent lack of filling of small vessels with the contrast agent was secondary to a dilutional phenomenon. This possibility is unlikely in view of the fact that many small vessels adequately filled distal to the injury site and the expansion of the intravascular volume was transient.

It can be suggested that the improved fluorescent vascular pattern in the mannitol-treated spinal cords may actually represent the perfusion of more vessels, but the rate of flow may be decreased. A decreased perfusion pressure could account for a partial collapse of the microvasculature. This partial collapse would not be extensive enough to inhibit the passage of Thioflavine S but could be sufficient enough to prohibit the passage of the barium suspension (particle size of 0.1 to 0.5 microns). An analysis of blood pressures between these two groups revealed no significant changes, though mannitol-treated animals tended

to show a mild improvement over the untreated animals in terms of blood pressures. In addition, barium was always infused at the pretrauma blood pressure which was either equal if not greater than the pre-infusion blood pressure.

It was concluded that in the mannitol-treated animals, Thioflavine S had demonstrated blood flow in vessels which could not be filled with micropaque-gel suspension. The distribution pattern in the microangiography series was a function of the vessels that it filled. The degree of filling with contrast appears to be dependent on many factors. Particle size, time length of infusion, concentration, temperature, infusion pressure, as well as the patency of the vessels govern the degree of filling.[63] These factors did not vary in this study. The extravasation of contrast material was interpreted to be solely the result of contrast being forced through holes in torn vessels and/or the instability of a formed thrombus, and did not correlate well with the size of the central hematoma.

Studies employing microangiography are injection experiments; to indicate that spasm was present would be erroneous, since this could not be actively demonstrated. The more appropriate term for this study is "vascular narrowing" instead of "spasm." It may be suggested that in future experiments it would be possible for other observers to complete such studies utilizing contrast media and magnification techniques and spasm may be demostrated.

The narrowing that was seen anatomically was postulated to be secondary to active vasospasm.

Because of the inconsistencies in the microvascular patterns in the spinal cords previously tested with mannitol and studied by microangiography and those studied by Thioflavine S technique, it was postulated that some physical or chemical reaction between the mannitol and the micropaque-gel suspension was occurring at the injury site. The fact that mannitol is rapidly excreted and that this effect was seen at a time when no intravascular mannitol should be present suggests that this may not be a direct reaction. The role of possible extravasated mannitol at the injury site is unclear apropos of the unusual microangiographic pattern in this group.

There is no knowledge of previous studies employing this microangiographic technique following mannitol therapy. Whether microangiography will be precluded by mannitol therapy as a means of assessing microvascular status following trauma will be dependent upon future studies that investigate particle size, infusion time, concentration and infusion pressures as variables. Experiments of this nature may also direct the way to optimal suspensions and modes of administration of perfusates that insure maximum visualization of the spinal cord vasculature under traumatic conditions; they may also assist in the practical application of in vivo angiography to experimental spinal cord trauma.

Summary

The effects of mannitol on the alterations in spinal cord blood flow patterns in experimental traumatic paraplegia were correlated with microangiographic studies. Spinal cord blood flow patterns were studied using Thioflavine S, a fluorescent dye that stains the endothelium of blood vessels through which it has passed. A micropaque-gel suspension was used to study vascular structure using high resolution microangiographic techniques. Feline spinal cords, exposed by laminectomy at the T-5 level, were traumatized with a 500 gm-cm contusion, a force sufficient to render the animal permanently paraplegic.

Microangiography proved to be an effective means of evaluating the morphology of the microvasculature of the spinal cord. The correlation of microangiography with a more dynamic study using fluorescent blood flow patterns generated much more useful information about the vascular changes occurring within the spinal cord following trauma than with microangiography alone. The data suggests that difficulty may be encountered in the use of microangiography following mannitol therapy in the traumatized spinal cord.

In the spinal cord-injured animals(500 gm-cm contusion) the cortical evoked response disappeared and did not return during the four hours post-trauma in both the untreated and mannitol-treated groups. At one hour following a

therapeutic dose of mannitol (3 gm/kg) an improved fluorescent vascular pattern was detected among the mannitol-treated spinal cords relative to the untreated spinal cords. By four hours perfusion of the lateral white matter approximated normal in the mannitol-treated spinal cords. This pattern of perfusion was in striking contrast to the spinal cords of untreated animals which displayed a near total lack of fluorescing vessels at this time period. These findings correlated with an increased vascular caliber, which was postulated to be the result of a decrease in vasospasm among the mannitol-treated spinal cords with microangiography. The increased perfusion detected by blood flow pattern studies and the increased vascular caliber noted by microangiography was postulated to be the result of vasodilation and the resulting expanded intramedullary blood volume following mannitol therapy.

In the untreated and mannitol-treated spinal cords the perfusion of the posterior columns remained markedly depressed over four hours and the central gray matter remained in a state of hemorrhagic non-perfusion. These changes were felt to be irreversible and the result of disruptive forces of the initial mechanical impact.

The absence of extravasation of contrast material and the poor filling of the arterial network of the central gray matter and white matter in mannitol-treated

spinal cords was postulated to be the result of a physical or chemical reaction between the micropaque-gel suspension and mannitol at the injured site, since normal filling in non-traumatized segments was observed. Blood flow studies demonstrated an improved perfusion pattern. These inconsistencies attest to the difficulty in assessing distribution patterns employing a passive microvascular injection.

The finding of markedly improved spinal cord blood flow patterns following mannitol therapy is encouraging. More controlled studies that assess the efficacy of mannitol in functional recovery following trauma are suggested. Appropriate trauma dose-response curves should be derived. Optimal times of administration of mannitol should be known. Continued investigation of the pathophysiological and vascular events following spinal cord trauma is necessary if more effective methods of treating the spinal cord-injured patient are to be found.

Table 1

Degree of Vascular Filling

Hours after trauma	White Matter		Gray Matter		Border Zone	
	U	T	U	T	U	T
Control	++++	++++	++++	++++	++++	++++
1.5	++	+	+	++	+	+
2	+	+	+	++	+	+
3	+++	+	+	+	++	+
4	++	+	+	+	++	+

U = untreated; T = mannitol-treated
++++ = normal; +++ = mildly decreased; ++ = moderately decreased; + = severely decreased

Table 2

Degree of Extravasation

Hours after trauma	Control	1.5	2	3	4
U	0	2	3	2	1
T	0	0	0	1	1

U= untreated, without mannitol; T= treated with mannitol; 0= absent; 1= mild, small focal areas; 2= moderate, beginning to coalesce; 3=severe, encompassing entire gray matter.

Table 3

Degree of Vascular Narrowing

Hours after trauma	Control	1.5	2	3	4
U	0	3	1	0	0
T	0	1	0	1	0

U=untreated, without mannitol; T=treated with mannitol; 0=absent; 1=mild; 2=moderate; 3=severe.

Illustrations

Figure No. 1

Figure No. 2

Figure 1. Placement of electrodes. After placing the animal in a stereotaxic frame, a midline scalp incision was made and the coronal sutures identified. Screw electrodes were placed in the right and left frontal skull so as to be over the primary somatosensory area. Reference electrodes were placed in the frontal sinuses.

Figure 2. Experimental model set-up. After laminectomy was performed, the spinal cord was traumatized. A 20 gm weight was dropped a distance of 25 cm through a perpendicularly oriented plastic tube directly onto an impounder resting on the spinal cord.

Figure 1.

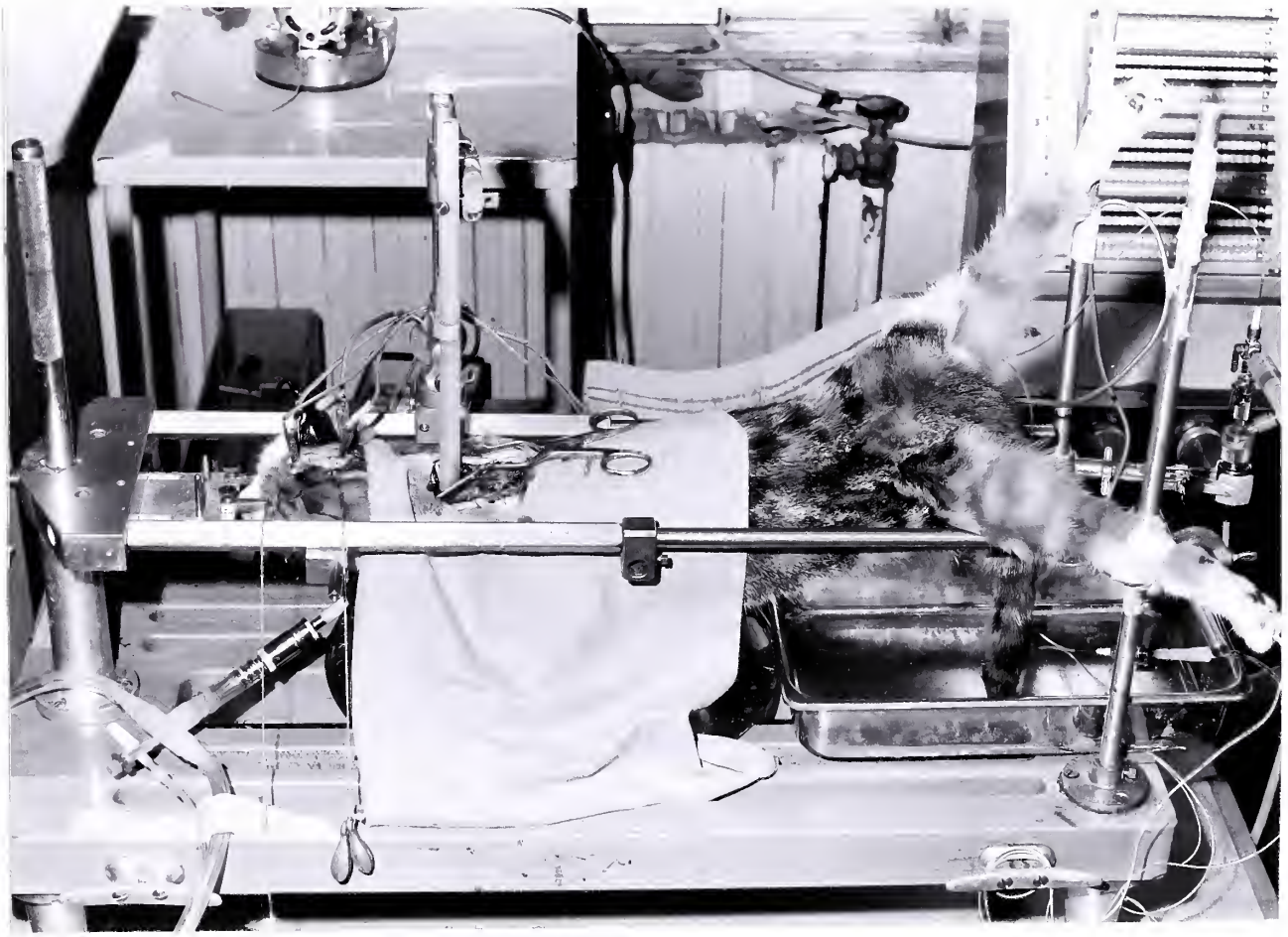


Figure 2.

Figure No. 3

Figure No. 4

Figure 3. Spinal cord specimen sealed in plastic. After ten days of fixation the spinal cord segment was dissected from the vertebral column and the dura removed. The specimen was sealed in a commercial plastic to prevent drying and then radiographed in the anteroposterior and lateral projections. Cross-sections, 3 mm in thickness were subsequently radiographed in a similar manner.

Figure 4. X-ray apparatus. The radiographic unit consisting of a water-cooled type 0-2L diffraction tube with a thin beryllium window and a chromium-copper target with a 0.2 x 1.0 mm focal spot. The extension cone allowed a 70 cm focus-to-field distance and was fitted at the bottom with a casette receiver.

Figure 3.

Figure 4.

Figure No. 5

Figure 5.A. Representative blood pressure changes following a 500 gm-cm contusion to the thoracic spinal cord in the untreated control. Note the pressor response immediately after impact and the hypotensive phase which follows the hypertensive phase.

Figure 5.B. Representative blood pressure changes following a 500 gm-cm contusion to the thoracic spinal cord in the mannitol-treated animal. Note the widened pulse pressure and increased systolic and diastolic pressure which follows mannitol infusion.

Untreated

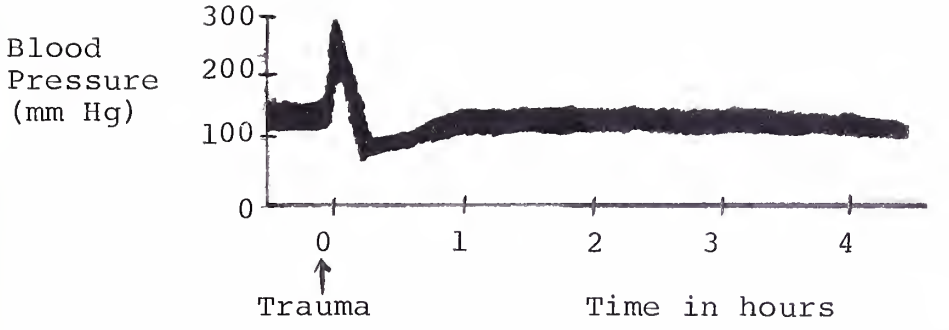


Fig.5.A.

Mannitol-Treated

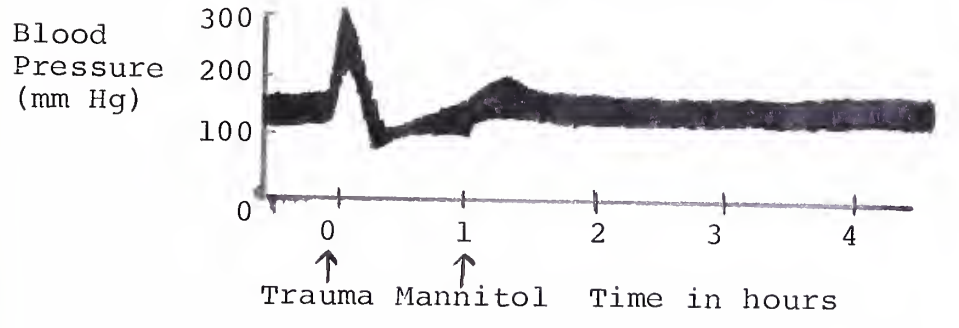


Fig.5.B.

Figure No. 6

Figure 6.A. Cortical evoked response pathway(dotted line). Impulses from stimulation of peripheral nerve enter the posterior root, then enter posterior columns, ascend and cross to the other side of the neuraxis and synapse in the VPL before projecting to the somatosensory cortex.

Figure 6.B. Control response representative of the averaged evoked responses from the somatosensory cortex to stimulation of the contralateral posterior tibial nerve prior to 500 gm-cm contusion. a. stimulus artifact. b. evoked cortical response (CER).

Figure 6.C. Representative averaged evoked response four hours after injury. Note the complete absence of the CER.

Figure 6.A.

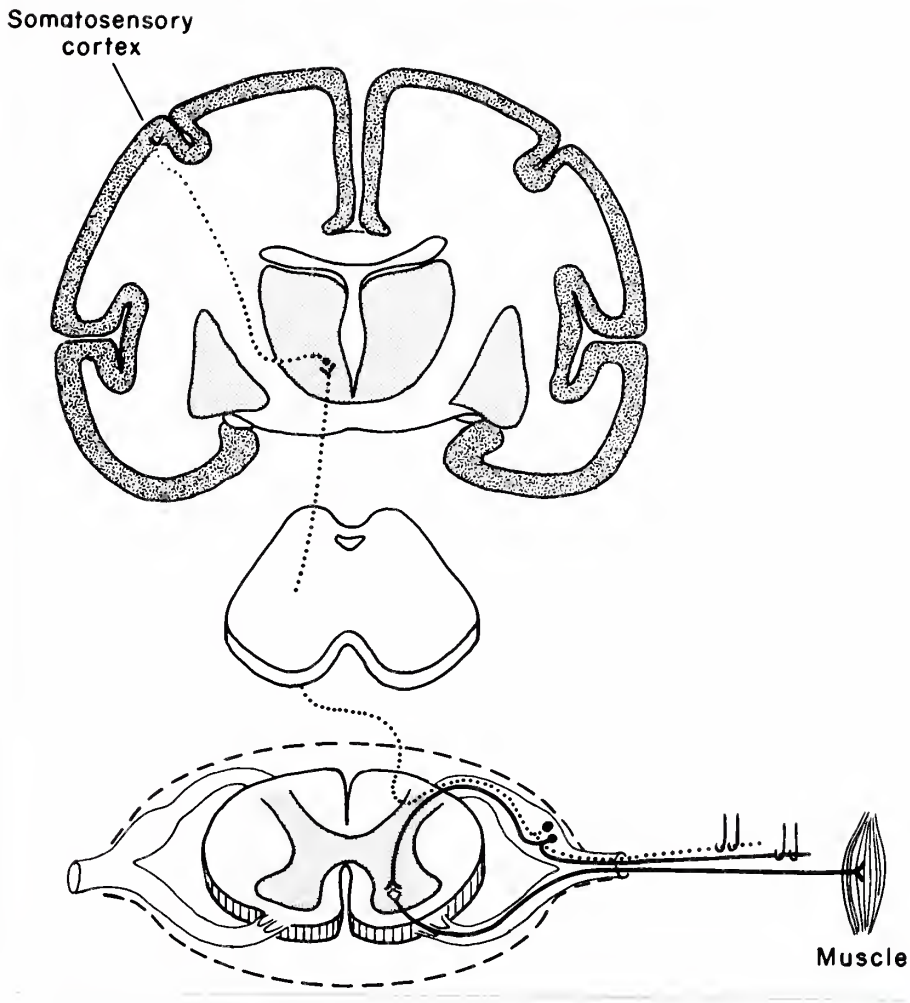


Figure 6.B. Pretrauma

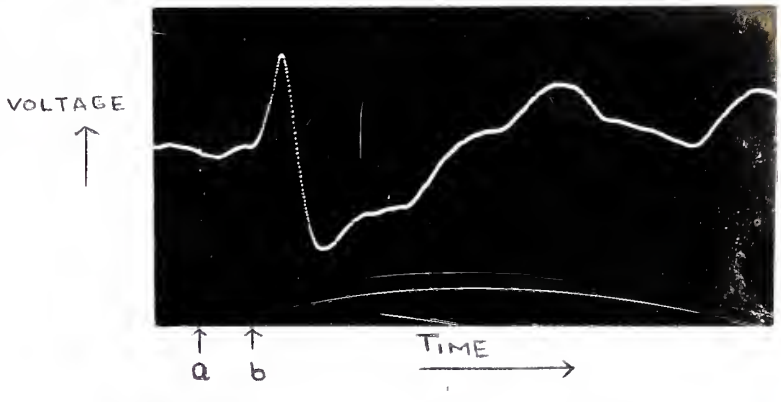


Figure 6.C. Four hours post-trauma

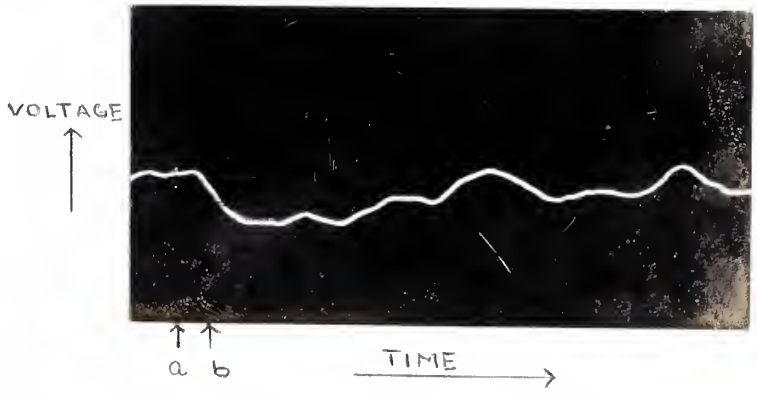


Figure No. 7

Figure 7.A. Normal microvasculature of the feline spinal cord. Anteroposterior projection. Note the rich anastomotic network over the pial surface arising from the spinal arteries.

Figure 7.B. Lateral projection. The tortuous central arteries can be seen arising from the anterior spinal artery.

Figure 7.C. Cross-section. A relatively sparse supply to the white matter arises from the peripheral arteries. The central artery gives rise to a rich arterial network to the anterior and lateral gray matter. The posterior gray matter is supplied by large, penetrating branches from the posterior spinal arteries. There is an overlapping supply to the peri-gray white matter, especially in the region of the anterior horns. The insert as a contact print represents the actual size of this specimen.

Figure 7.A.

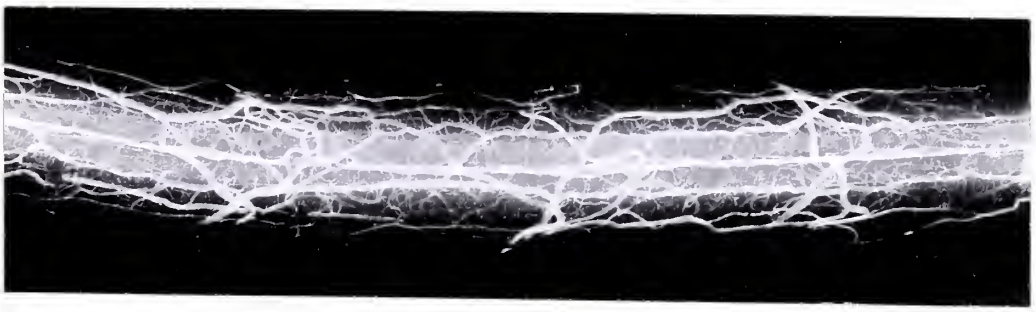


Figure 7.B.

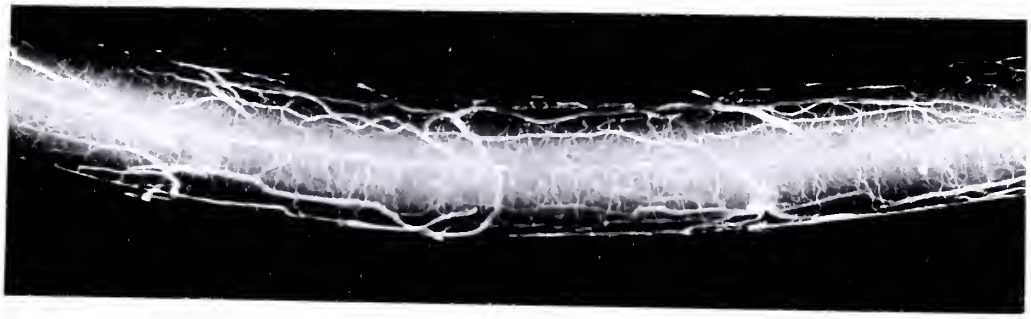


Figure 7.C.

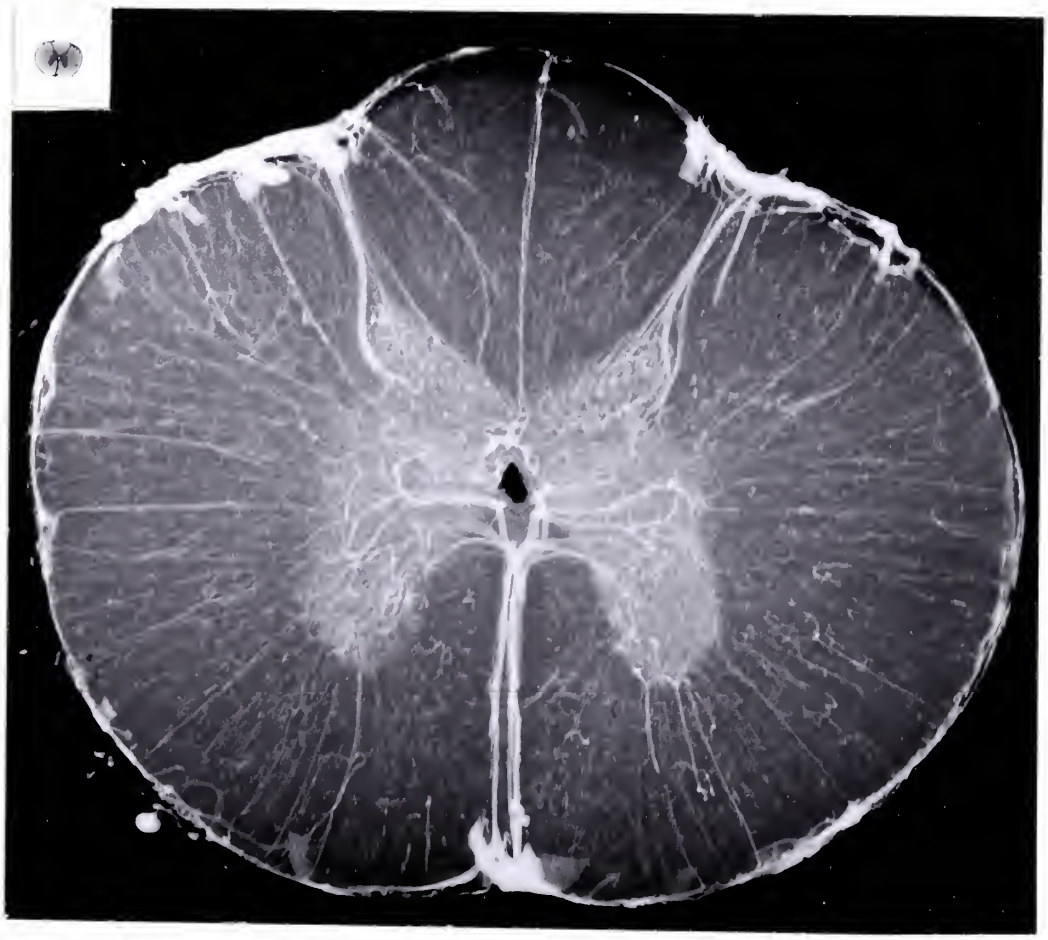


Figure No. 8

Figure 8.A. One and one-half hours post-trauma. Antero-posterior. Note the severe narrowing of the posterior spinal arteries and to a much lesser extent the anterior spinal artery. A=anterior spinal artery; P=posterior spinal artery.

Figure 8.B. One and one-half hours post-trauma. Cross-section. Note perforating branches of posterior spinal artery are moderately decreased in number and the severe narrowing of vessels throughout the gray matter; also note the moderate focal extravasation of contrast. Note the moderate paucity and diffuse severe narrowing of the peripheral vessels.

Figure 8.A.

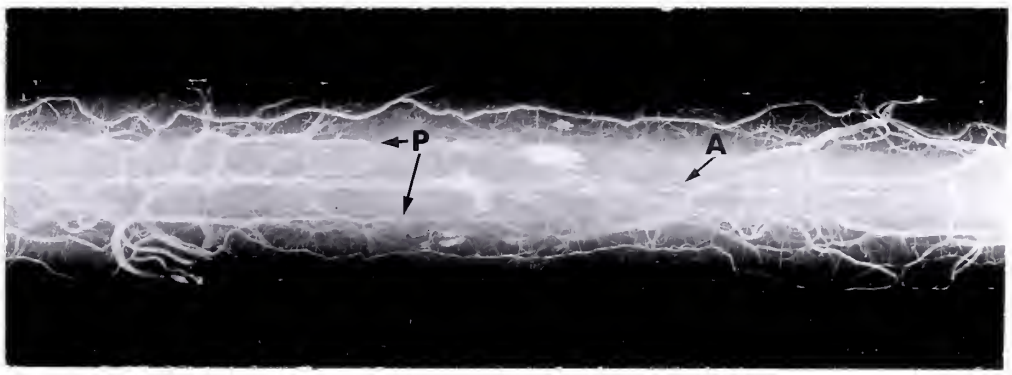


Figure 8.B.

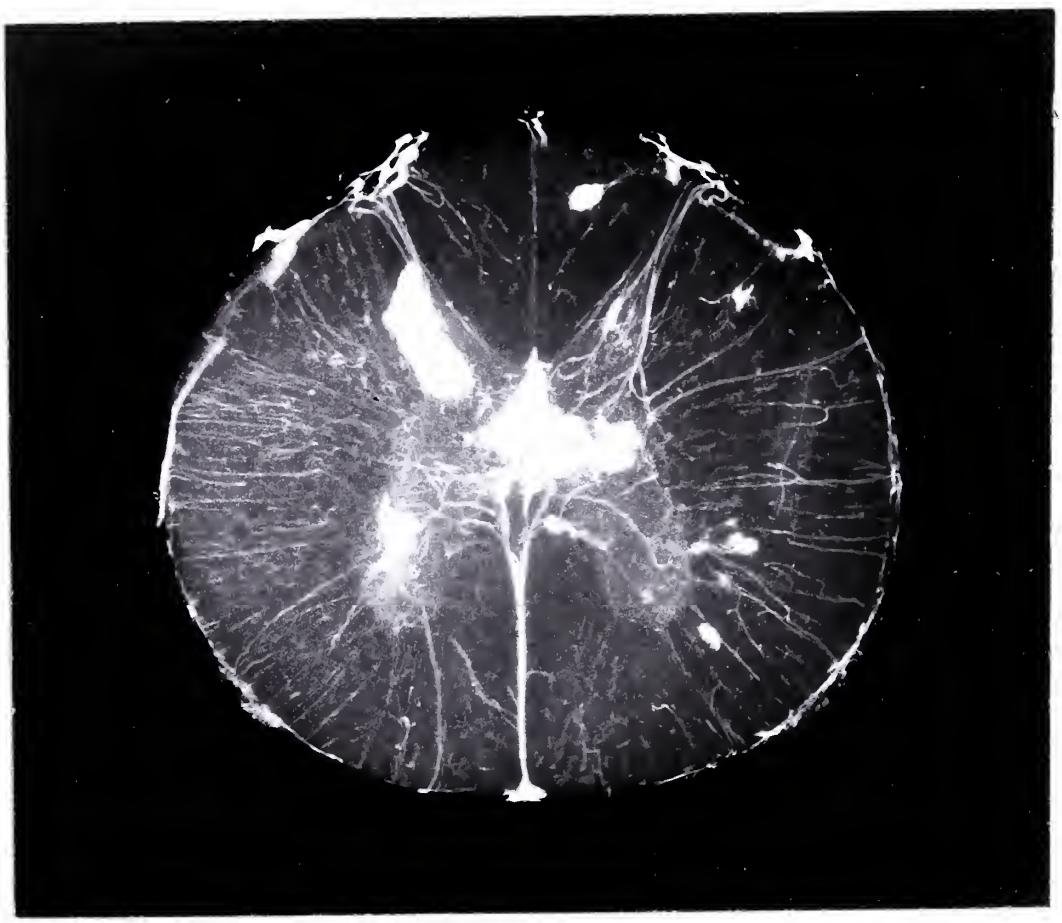


Figure No. 9

Figure 9.A. Two hours post-trauma. Anteroposterior. The caliber of the surface vessels is much improved over the 1½ hour group. There is further extravasation and coalescence of contrast material.

Figure 9.B. Lateral. The perforating branches of the posterior spinal vessels are decreased in number and display a mild degree of narrowing.

Figure 9.C. Cross-section. Note that the central gray matter is practically replaced by contrast material and there is essentially no filling of the peripheral vessels supplying the white matter.

Figure 9.A.

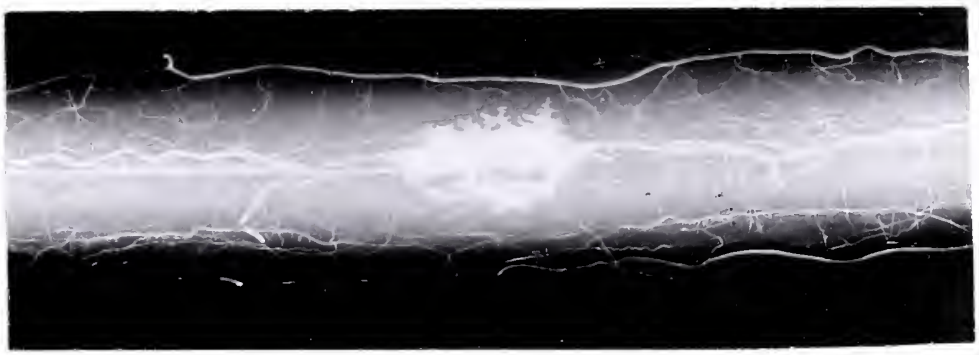


Figure 9.B.

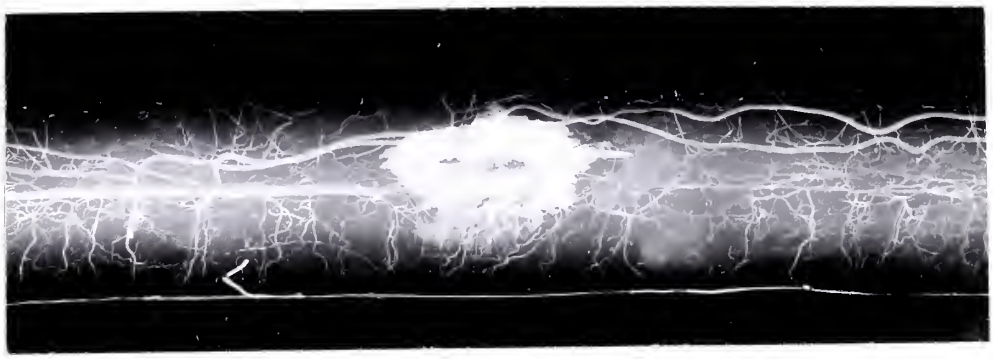


Figure 9.C.

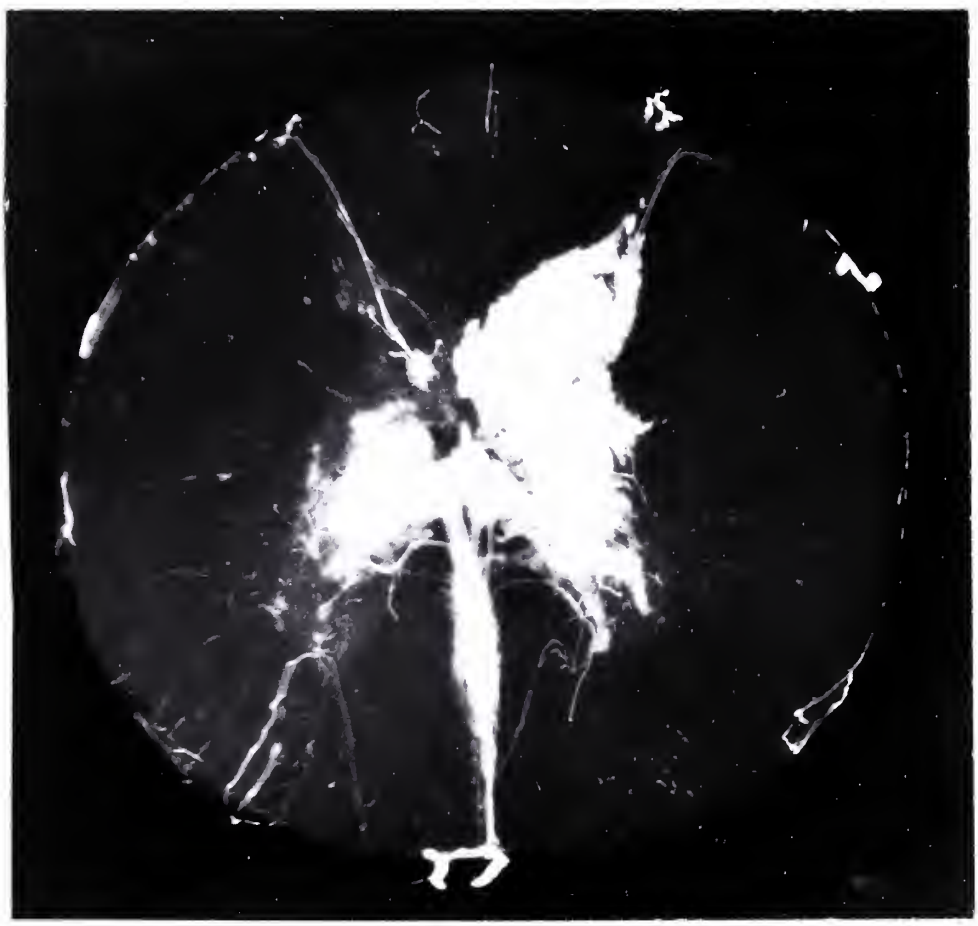


Figure No. 10

Figure 10.A. Three hours post-trauma. Anteroposterior. There is comparatively less extravasation of contrast material; however, a paucity of intramedullary vessels persists.

Figure 10.B. Note the swelling of the spinal cord at the injury site.

Figure 10.C. Cross-section. Note the focal areas of extravasated contrast throughout the gray matter and the mild narrowing of the penetrating branches of the posterior spinal arteries. The peripheral vasculature approached a normal distribution, though reduced in caliber.

Figure 10.A.

Figure 10.B.

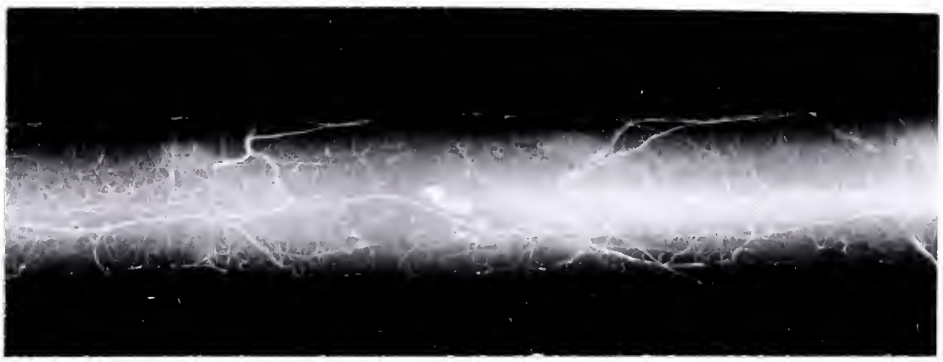


Figure 10.C.

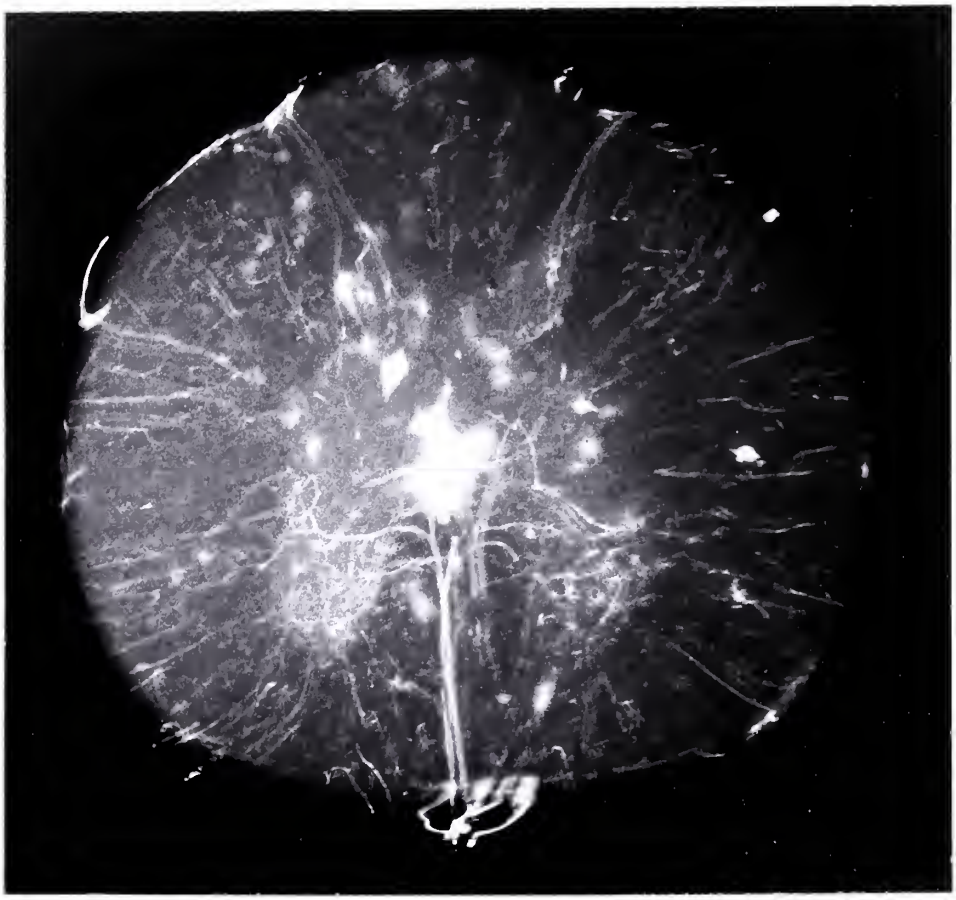


Figure No. 11

Figure 11.A. Four hours post-trauma. Anteroposterior. The surface vessels appear normal in caliber and there remains a relative decrease in filling of the intramedullary vessels at the site of trauma.

Figure 11.B. Lateral. Note the swelling at the trauma site.

Figure 11.C. Cross-section. Note that only the major branches of the central and posterior spinal arteries fill and the absence of extravasation.

Figure 11. A.

Figure 11.B.

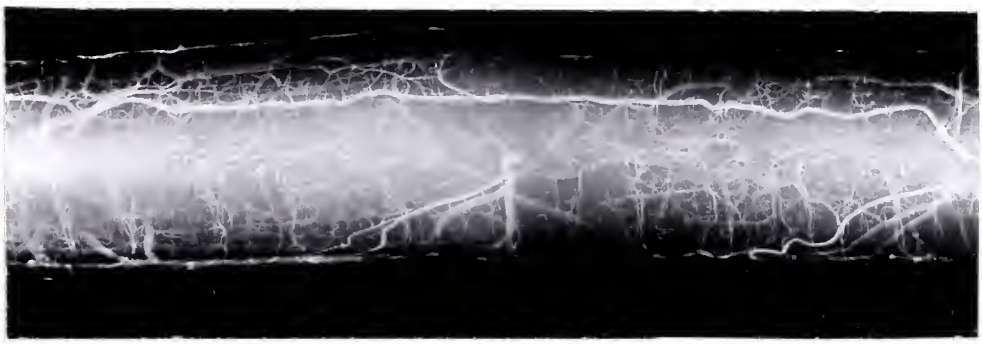


Figure 11.C.

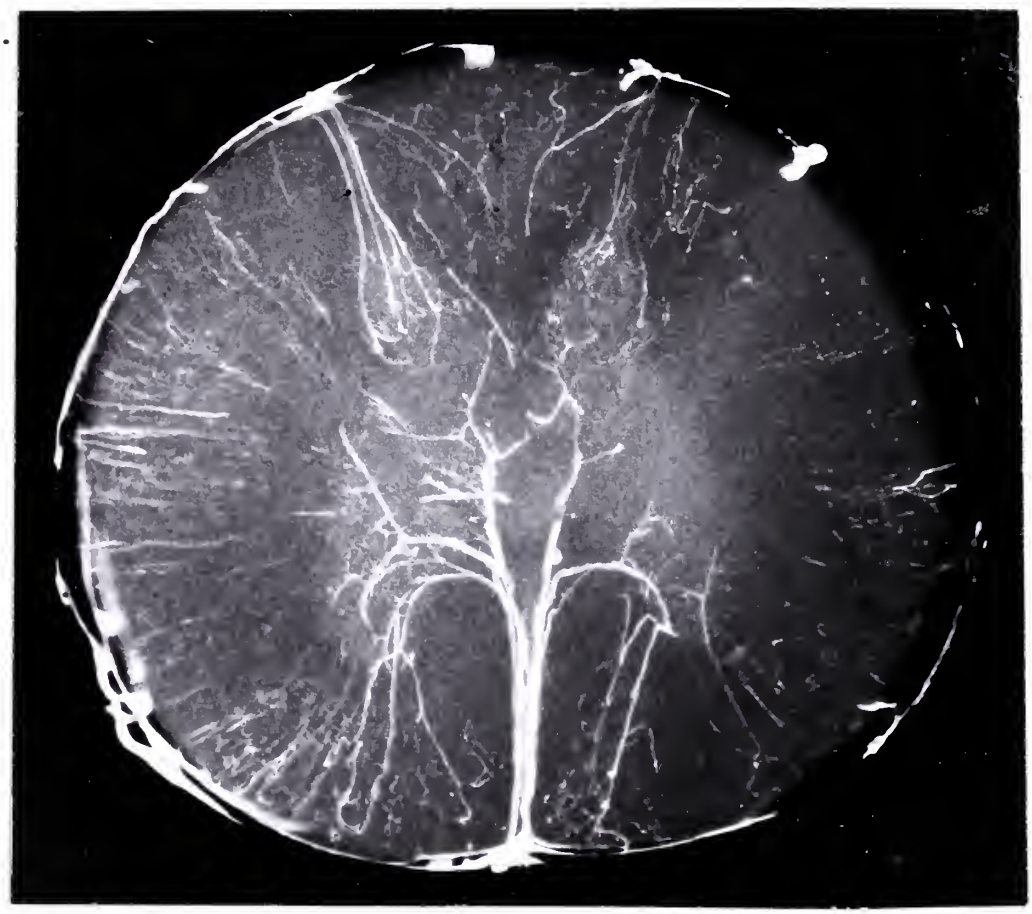


Figure No. 12

Figure 12.A. One and one-half hours post-trauma(30 minutes after mannitol). Anterorposterior projection. Note the almost normal cailber of the vasculature.

Figure 12.B. Lateral. Note the small collection of contrast at the end of one of the major branches of the central artery.

Figure 12.C. Cross-section. Only the major vessels of the anterior and posterior spinal vessels filled. Note the marked paucity of the peripheral vasculature.

Figure 12.A.

Figure 12.B.

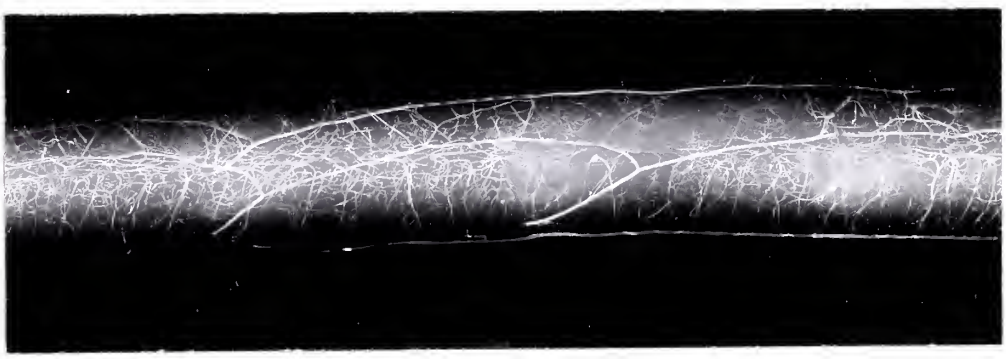


Figure 12.C.

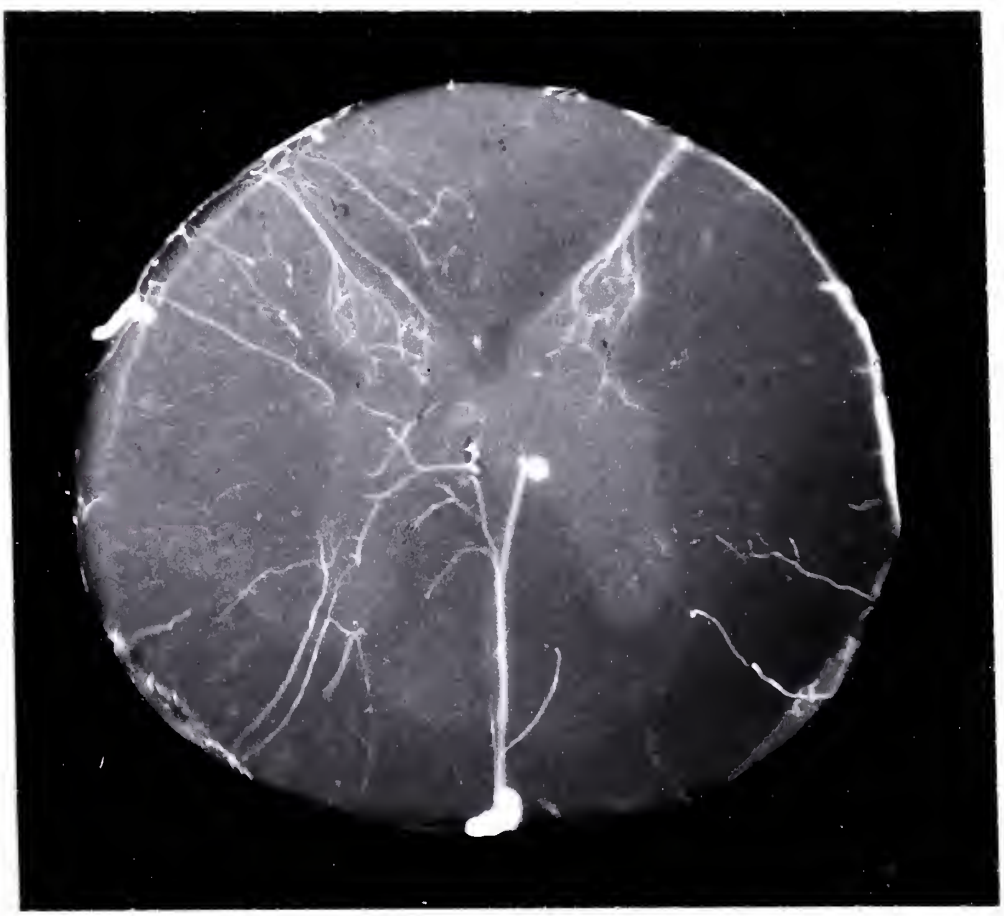


Figure No. 13

Figure 13. Two hours post-trauma (one hour post-mannitol). Cross-section. Note that only the major vessels of the posterior and anterior spinal arteries fill. Note the absence of vascular narrowing, extravasation and filling of the vessels of the white matter.

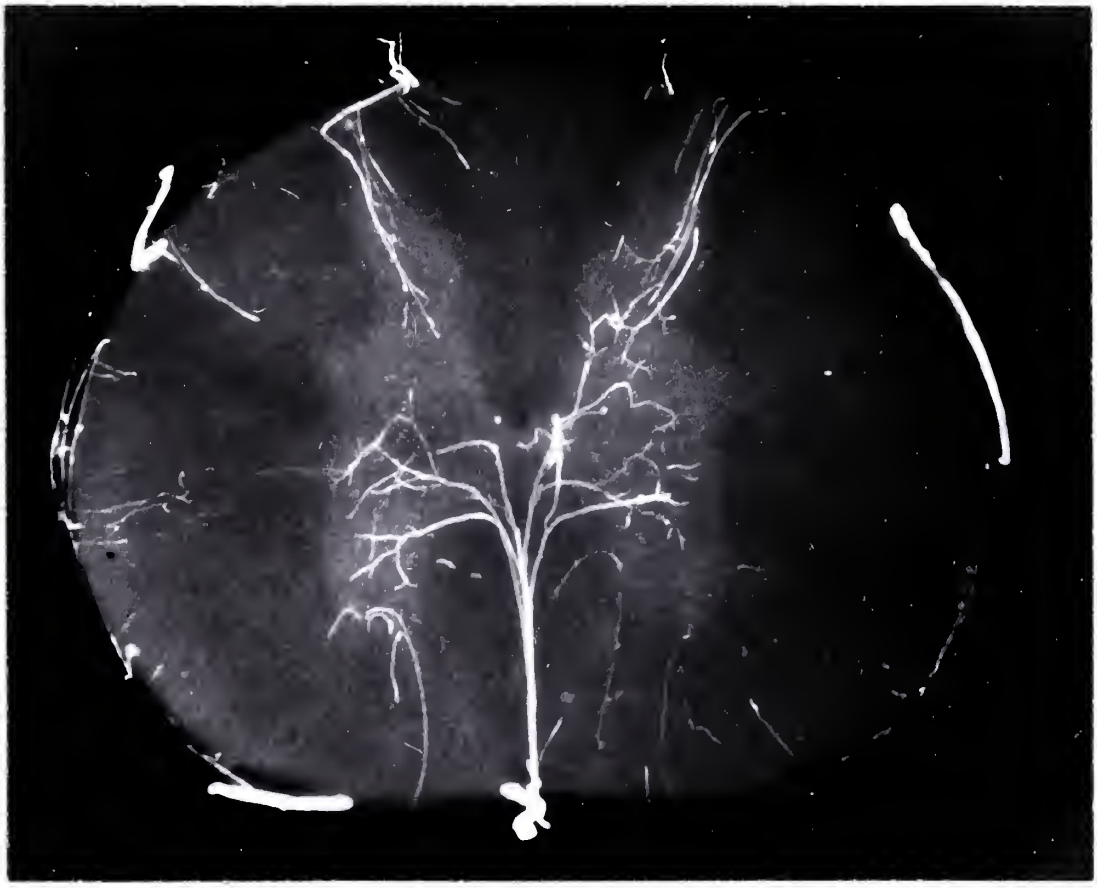


Figure 13.

Figure No. 14

Figure 14.A. Three hours post-trauma (two hours post-mannitol). Anteroposterior projection. Note the mild degree of vascular narrowing at the trauma site of the anterior and posterior spinal arteries.

Figure 14.B. Lateral. Note the small collection of contrast at the end of the branch of the central artery.

Figure 14.C. Cross-section. Note that there is very little filling of the peripheral white matter vasculature.

Figure 14.A.

Figure 14.B.

Figure 14.C.

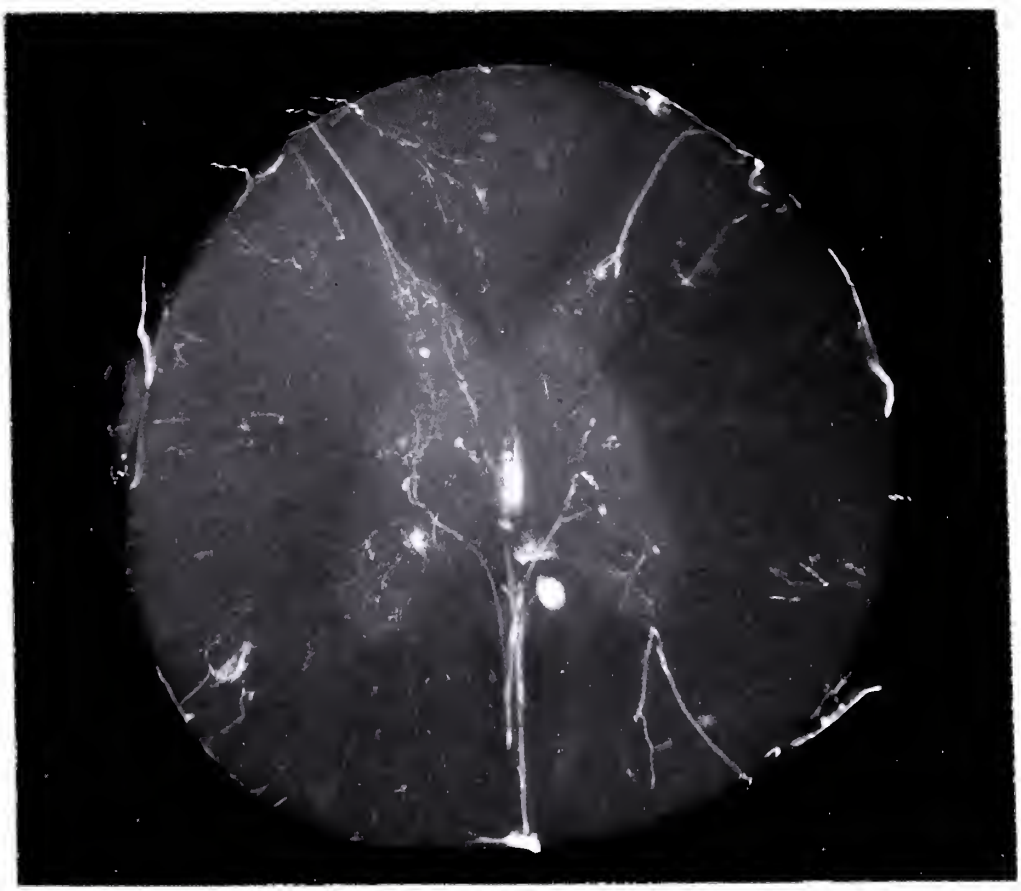


Figure No. 15

Figure 15.A. Four hours post-trauma (three hours post-mannitol). Anteroposterior projection. Note that the surface vessels are of normal caliber.

Figure 15.B. Lateral. Note the small coalescence of contrast centrally.

Figure 15.C. Cross-section. Note that there is slightly more filling of the peripheral system. More perforating branches of the anterior and posterior spinal arteries are seen.

Figure 15.A.

Figure 15.B.

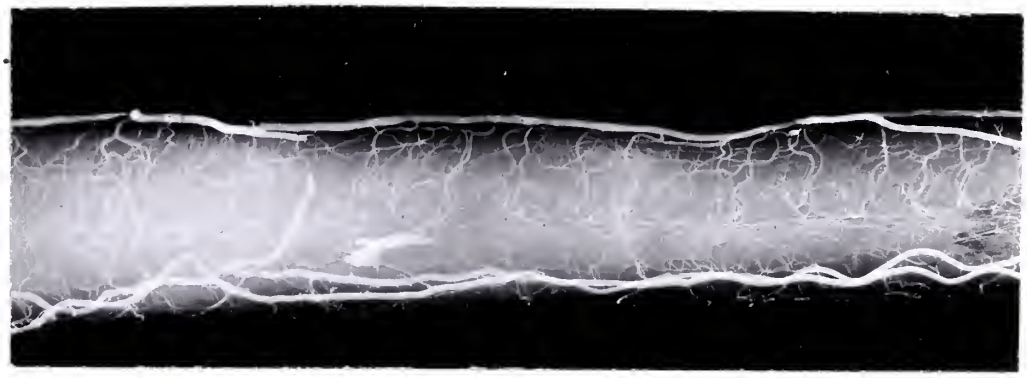


Figure 15.C.

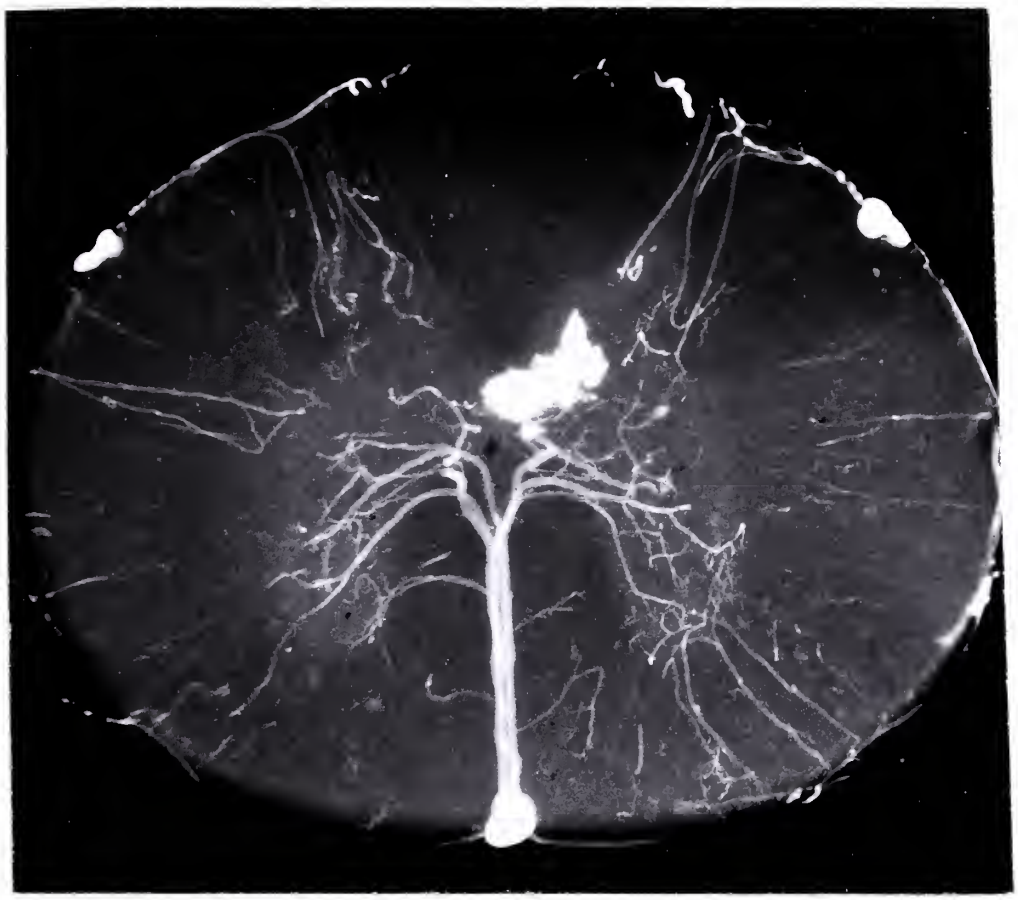
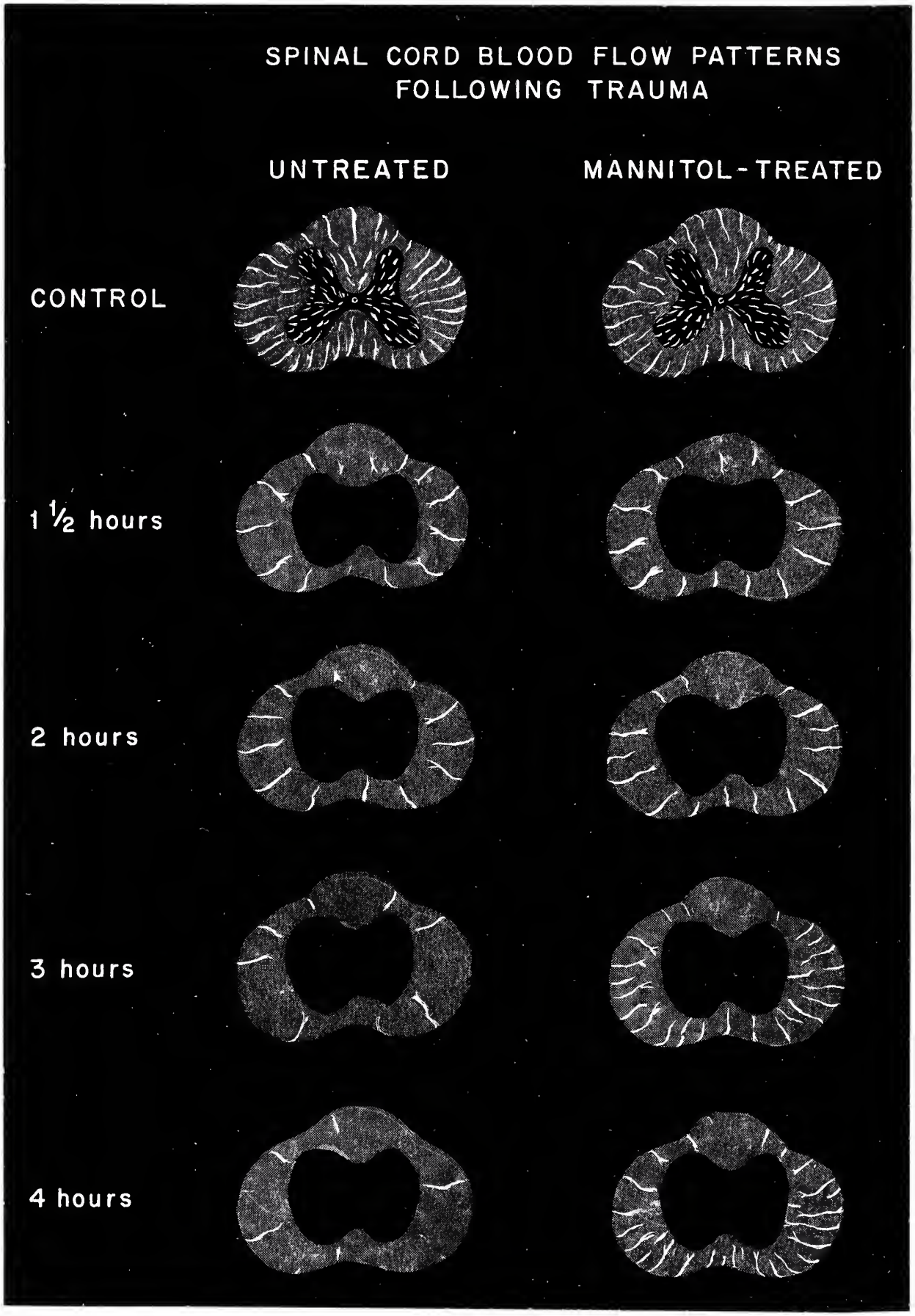


Figure No. 16

Figure 16. Line drawing showing representative blood flow patterns in untreated and mannitol-treated spinal cords following 500 gm-cm contusion. Mannitol was infused one hour following trauma in the mannitol-treated animals. Note the slightly improved fluorescent vascular pattern among the mannitol-treated spinal cords at two hours. Note that the mannitol-treated spinal cords progressively improved so that by four hours a perfusion pattern in the lateral white matter approximated normal. Note the contrast with the four hour untreated spinal cord.


SPINAL CORD BLOOD FLOW PATTERNS
FOLLOWING TRAUMA
UNTREATED
MANNITOL-TREATED
CONTROL
1 ½ hours
2 hours
3 hours
4 hours

Bibliography

1. Albin, M.S., White, R.J., Acosta-Rua, G., et al.: Study of functional recovery produced by delayed cooling after spinal cord injury in primates. J Neurosurg. 29:113-120, 1968.

2. Albin, M.S., White, R.S., Yashon, D., et al.: Effects of localized cooling in spinal cord trauma. J Trauma 9:1000 - 1008, 1969.

3. Alexander, S., and Kerr,F: Blood pressure responses in acute compression of the spinal cord. J Neurosurg. 2:485-491, 1964.

4. Allen, A.R: Remarks on the histopathological changes in the spinal cord due to impact. An experimental study. J Nerv. Ment. Dis.41:141-147, 1914.

5. Allen, A.R: Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column; a preliminary report. J.A.M.A. 57:878-880, 1911.

6. Allen, W.E.III, D'Angelo, C.M. and Kier, E.L: Correlation of microangiographic and electrophysiologic changes in experimental spinal cord trauma. Radiology 111:107-115, 1974.

7. Ashford, T., Palmerio, C., Fine, J: Structure analogue in vascular muscle to the functional disorder in refractory traumatic shock and reversal by corticosteroid: electron microscopic evaluation. Ann. Surg. 164:575-585, 1966.

8. Assenmacher, D.R., Ducker,T.B: Experimental Traumatic Paraplegia. The vascular and pathological changes seen in reversible and irreversible spinal cord lesions. J. Bone Joint Surg. 53A:671-680, 1971.

9. Bakay, L., Lee, J: Cerebral Edema, Bannerstone House, Springfield, Illinois, 1965.

10. Breasted, J.H: A crushed cervical vertebrae. The Edwin Smith Surgical Papyrus I:337-342, 1930.

11. Brodner, R.A., Dohrmann, G., Rubin, R.A: Cerebrospinal fluid and spinal cord serotonin: correlation with intramedullary blood flow patterns in experimental spinal cord trauma (research in progress). Presented at the 5th

European Congress of Neurology, Oxford, England, Sept. 19, 1975.

12. Campbell, J.L., DeCrescito, V., Tomasula, J.J. et al: Experimental treatment of acute spinal cord contusion. Presented to the 40th Annual Meeting of the American Association of Neurological Surgeons, Boston, April 1972.

13. D'Angelo, C.M: The H-reflex in experimental spinal cord trauma. J Neurosurg. 39:209-213, 1973.

14. D'Angelo, C.M., Van Gilder, J.C., and Taub: Evoked cortical potentials in experimental spinal cord trauma. J Neurosurg. 38:332-336, 1973.

15. De La Torre, J.C., Johnson, C.M., Harris, L.H., et al: Monoamine changes in experimental head and spinal cord trauma: failure to confirm previous observations. Surg. Neurol. 2:5-11, 1975.

16. Dohrmann, G.J., Allen, W.E.III: Microcirculation of traumatized spinal cord: a correlation of microangiography and blood flow patterns in transitory and permanent paraplegia. J Trauma 15:1003-1013, 1975.

17. Dohrmann, G.J., and Wagner, F.C.Jr.: Combination of electron microscopy and the Thioflavine S technique in studies of the spinal cord microvasculature. Microvasc. Res. 10:228-233, 1975.

18. Dohrmann, G.J., Wagner, F.C., and Bucy, P.C: The microvasculature in transitory traumatic paraplegia: an electron microscopic study in the monkey. J Neurosurg. 35:263-271, 1971.

19. Dohrmann, G.J., Wagner, F.C., and Bucy, P.C: Transitory traumatic paraplegia: electron microscopy of early alterations in myelinated nerve fibers. J Neurosurg. 36:407-415, 1972.

20. Dohrmann, G.J., and Wick, K.M: Demonstration of the microvasculature of the spinal cord by an intravenous injection of the fluorescent dye, Thioflavine S. Stain Technol. 46:321-322, 1971.

22. Dohrmann, G.J., and Wick, K.M: Intramedullary blood flow patterns in transitory trauma paraplegia. Surg. Neurol. 1:209-215, 1973.

23. Donaghy, R.M.P., and Numoto, M: Prognostic significance of sensory evoked potential in spinal cord injury. Proc. Ann. Clin. Spinal Cord Inj. Conf. 17:251-257, 1969.

24. Doppman, J.L., Ramsey, R., and Thies, R.J: A percutaneous technique for producing intraspinal mass lesions in experimental animals. J Neurosurg. 38:438-447, 1973.

25. Ducker, T.B., and Assenmacher, D: Microvascular response to experimental spinal cord trauma. Surg. Forum 20:428-430, 1969,

26. Ducker, T.B., and Assenmacher, D: The pathological circulation in experimental spinal cord injury. Proc. Ann. Clin. Spinal Cord Inj. Conf. 17:10-11, 1969.

27. Ducker, T.B., Hamit, H.F: Experimental treatments of acute spinal cord injury. J Neurosurg. 30:693-697, 1969.

28. Ducker, T.B., Kindt, G.W., and Kempe, L.G: Pathological findings in acute experimental spinal cord trauma. J Neurosurg. 35:700-708, 1971.

29. Ducker, T.B., and Kindt, G.W: The vasomotor control of the spinal cord circulation. Proceedings of the 17th Spinal Cord Injury Conference, Sept. 1969, pp.69-70.

30. Ducker, T.B., and Perot, P.L.,Jr.: Spinal cord oxygen and blood flow in trauma. Surg. Forum 22:413-415, 1971.

31. Eidelberg, E: Cardiovascular response to experimental spinal cord compression. J Neurosurg. 38:326-331, 1973.

32. Eidelberg, E: Cardiovascular response to experimental spinal cord compression. J Neurosurg. 38:326-331, 1973.

33. Fairholm, D.J., and Turnbull, I.M: Microangiographic study of experimental injuries in dogs and rabbits. Surg. Forum 21:453-455, 1970.

34. Fairholm, D., and Turnbull,I.M: Microangiographic study of experimental spinal cord injuries. J Neurosurg. 35:277-286, 1971.

35. Ferraro, A: Experimental medullary concussion of the spinal cord in rabbits. Histological study of the early stages. Arch. Neuro. Psychiat. 18:357-373, 1927.

36. Fried, L.C: Progress in Spinal Cord Injury Research Program Analysis Report #19. National Institute of Health, Dept. of Health, Education and Welfare, May 1968.

37. Goodkin, R., and Campbell, J.B: Sequential Pathological changes in spinal cord injury. A preliminary report. Surg. Forum 20:430-432, 1969.

38. Green, B.A., and Wagner, F.C: Evolution of edema in the acutely injured spinal cord: a fluorescence microscopic study. Surg. Neurol. 1:98-101, 1973.

39. Griffiths, J.R: Sinal cord blood flow in dogs: the effect of blood pressure. J Neurol., Neurosurg. and Psychiat. 36:914-920, 1973.

40. Hedeman, L.S., Shellenberger, M.K., Gordon, J.H: Studies in experimental spinal cord trauma. Part 1 Alterations in catecholamine levels. J Neurosurg. 40:37-43,1974.

41. Joyner, J., Freeman, L.W: Urea and spinal cord trauma. Neurology 13:69-72, 1963.

42. Kapp, J., Mahaley, M.S.Jr., Odom, G.L: Cerebral arterial spasm: Part 2: experimental evaluation of mechanical and humoral factors in pathogensis. J Neurosurg. 29:339-349, 1968.

43. Kelly, D.L., Lassiter, K.R.L., Calogero, J.A.,et al: Effects of local hypothermia and tissue oxygen studies in experimental paraplegia. J Neurosurg. 33:554-563, 1970.

44. Kelly, D.L., Lassiter, K.R.L., Vongsvivut, A., et al: Effects of hyperbaric oxygenation and tissue oxygen studies in experimental paraplegia. J Neurosurg. 36:425-429, 1972.

45. Kindt, G.W: Autoregulation of spinal cord blood flow. European Neurology 6:19-23, 1971.

46. Klatzo, I: Neuropathological aspects of brain edema. J Neuropath. and Exp. Neurol. 26:1-14, 1967.

47. Kormano, M., Reijonen: Microangiographic filling of the vascular system of the brain. Neurorad. 5:83-86, 1973.

48. Locke, G.E., Yashon, D., Feldman, R.A., et al: Ischemia in primate spinal cord injury. J Neurosurg. 34:614-617, 1971.

49. McVeigh, J.F: Experimental cord crushes with Especial reference to the mechanical factors involved and subsequent changes in the areas of the cord affected. Arch. Surg. 7:573-600, 1923.

50. Naftchi, N.E., Demeny, M., DeCrescito, V., et al: Biogenic amine concentrations in traumatized spinal cords of cats: effect of drug therapy. J Neurosurg. 40:52-57, 1974.

51. Osterholm, J.L: The pathophysiological response to spinal cord injury: the current status of related research. J Neurosurg. 40:5-33, 1974.

52. Osterholm, J.L., Bell, J., Meyer, R., et al: Experimental effects of free serotonin on brain and its relation to brain injury. Part 1: Neurological consequences of intracerebral serotonin injections. Part 2: Trauma-induced alterations in spinal fluid in brain. Part 3: Serotonin-induced cerebral edema. J Neurosurg. 31: 408-421, 1969.

53. Osterholm, J.L., and Mathews, G: Altered norepinephrine metabolism following experimental spinal cord injury. Part I: Relationship of hemorrhagic necrosis and post wounding neurological deficits. J Neurosurg. 36:386-394, 1972.

54. Osterholm, J.L., and Mathews, G: Altered norepinephrine metabolism following experimental spinal injury. Part II Protection against traumatic spinal cord hemorrhagic necrosis by norepinephrine synthesis blockade with with alpha-methyl tyrosine. J Neurosurg. 36:395-401, 1972.

55. Osterholm, J.L., Mathews, G: Spinal pathways mediating traumatic hemorrhagic necrosis. Trans Am. Neurol. Ass. 97:187-191, 1972.

56. Parker, A.J., et al: Reduction of trauma-induced edema of spinal cord in dogs given mannitol. Am. J. Vet. Res. 34:1355-1357, 1973.

57. Ramsey, R., and Doppman, J.L: The effects of epidural masses on spinal cord blood flow. Radiology 107:99-103, 1973.

58. Rawe, S.E., D'Angelo, C.M., Collins, W.F: The pressor response in experimental spinal cord trauma. Surg. Forum 25:432-433, 1974.

59. Richardson, H.D., Nakamaura, S: An electron microscopic study of spinal cord edema and the effect of treatment with steroids, mannitol and hypothermia. Proc. Veteran Adm. Spinal Cord Inj. Conf. 18:10-16, 1971.

60. Rosomoff, H.L: Effect of hypothermia and hypertonic urea on distribution of intracranial contents. J Neurosurg. 18:753-759, 1961.

61. Schmaus, H: Beiträge zur patholgischen Anatomie der Rückenmakkserschütterung. Virchows Arch. Path. Anat. 122:470-495, 1890.

62. Spiller, W.G: A critical summary of recent literature on concussion of the spinal cord with some original observations. Am. J. Med. Sci. 118:190-198, 1899.

63. Thompson, J.E: Pathological changes occurring in the spinal cord following fracture dislocation of the vertebrae. Ann. Surg. 78:260-293, 1923.

64. Turnbull, I.M: Microvasculature of the human spinal cord. J Neurosurg. 35:141-147, 1971.

65. Wagner, F.C.,Jr., Dohrmann, G.J., and Bucy, P.C: Histopathology of transitory traumatic paraplegia in the monkey. J Neurosurg. 35:272-276, 1971.

66. Wagner, F.C.Jr., Green, B.A., and Bucy, P.C: Spinal cord edema associated with paraplegia. Proc. Ann. Clin. Spinal Cord Inj. Conf., 1971.

67. Watson, B.A: An experimental study of lesions arising from severe concussions. Centralbl Allgem Pathol. 2:74, 1891.

68. White, R.J., Albin, M.S., Harris, L.S. et al: Spinal cord injury: sequential morphology and hypothermic stabilization. Surg. Forum 20: 432-434, 1969.

69. Wise, B.L., and Chater, N: The volume of hypertonic mannitol solution in decreasing brain mass and lowering cerebrospinal fluid pressure. J Neurosurg. 19:1038-1043, 1962.

70. Yashon, D., Bingham, G.Jr., Faddaul, E. et al: Edema of spinal cord following experimental impact trauma. J Neurosurg. 38:693-697, 1973.

71. Zielonka, J.S., Wagner, F.C. Jr., and Dohrmann, G.J: Alterations in spinal cord blood flow during local hypothermia. Surg. Forum 25:434-436, 1974.

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